THE SECOND OBSERVATIONAL STUDY OF CARDIO-VASCULAR RISK FACTORS IN THE GENERAL POPULATION IN LUXEMBOURG (ORISCAV-LUX 2)

A STATE-OF-THE-ART AND HYPOTHESIS-DRIVEN FOLLOW-UP STUDY

Research Unit Protocol Number: REC-CESP-20141206

Submission date: 15 April 2015

Funded by:

LIH - Ministry of Higher Education and Research

Author:

Dr. Ala'a AlKerwi, PhD, MD

Co-authors:

Dr. Gloria Aguayo, MD

Dr. Brice Appenzeller, PhD

Dritan Bejko, MSc

Dr Yvan Devaux, PhD

Marylène d'Incau

Dominique Mormont, PMP

Dr. Hanène Samouda, PhD

Centre for Health Studies – CES

Luxembourg Institute of Health – L.I.H.

SIGNATURE / APPROVAL PAGE

Project Investigator:

Signed: Ala'a Alkerwi, M.D., PhD Date: _____

Name

Title

Medical Investigator (if applicable):

Signed: Sophie Couffignal, M.D., Date: _____

Name

Title

Head of unit:

Signed: Saverio Stranges, M.D., PhD Date: _____

Name

Title

Key personnel

Name	Profile	Function	Laboratory / Institution
Gloria Aguayo	MD, MS	Researcher	CES, L.I.H.
Ala'a Alkerwi	MD, PhD	Senior Researcher, Project leader (PI)	CES, L.I.H.
Graziella Ambroset		Research nurse	CES, L.I.H.
Brice Appenzeller	PhD	Senior Researcher	LAHB, L.I.H.
Dritan Bejko	MSc	Scientific Collaborator	CES, L.I.H.
Agnès Columeau		Research nurse	CES, L.I.H.
Sophie Couffignal	MD	Associated Head of Centre d'Etudes en santé, Medical investigator	CES, L.I.H.
Yvan Devaux	PhD	Senior Researcher	Cardiovascular Research, L.I.H.
Marylène d'Incau		Senior research nurse, Fieldwork coordinator	CES, L.I.H.
Cathy Fousse		Administrative assistant	CES, L.I.H.
Melanie Kiemen		Research nurse	CES, L.I.H.
Maxime Larcelet		IT specialist	CES, L.I.H.
Dominique Mormont	PMP	CES Project manager	CES, L.I.H.
Jessica Pastore	BSc	Data manager	CES, L.I.H.
Hanène Samouda	PhD	Researcher	CES, L.I.H.
Nicolas Sauvageot	MSc	Statistician	CCMS, L.I.H.
Saverio Stranges	MD, PhD	Head of Population Health Department	L.I.H.

Abbreviations

AI	Augmentation index
ACL	Access control list
AUDIT	Alcohol use disorders test
CES-Depression	Center for Epidemiologic Studies Depression
CHD	Coronary heart disease
CHS	Cardiovascular health score
CSS	Constipation Scoring System
CVD	Cardiovascular disease
DASH	Dietary Approaches to Stop Hypertension
DII	Dietary inflammatory index
DQI-I	Diet Quality Index-International
ELSA	English Longitudinal Study of Ageing
CRF	Case Report Form
ICH	International Conference on Harmonization
L.I.H	Luxembourg Institute of Health
MDS	Mediterranean Diet Score
MetS	Metabolic syndrome
MHO	Metabolically healthy obese
MUO	Metabolically unhealthy obese
MHNW	Metabolically Healthy Normal Weight
MUNW	Metabolically Unhealthy Normal Weight
MHOV	Metabolically Healthy Overweight
MUOV	Metabolically Unhealthy Overweight

ORISCAV-LUX	Observation of cardiovascular risk factors in Luxembourg
PCR	Polymerase Chain Reaction
PP	Pulse pressure
PSQI	Pittsburg Sleep Quality Index- questionnaire
PWV	Pulse wave velocity
RCI	Recommendation Compliance Index
SAE	Serious adverse event
WHO	World Health Organisation

List of figures

LUXEMBOURG INSTITUTE OF HEALTH
RESEARCH DEDICATED TO LIFE

ORISCAV-LUX

Contents

1. PROJECT SUMMARY

1.1. Project abstract

Despite major advances in prevention, diagnosis and treatment measures, cardiovascular disease (CVD) remains the leading cause of hospitalization, disability and death worldwide, and in Europe, including Luxembourg. Moreover, recent research points out the negative cumulative impact of major CVD risk factors and associated pathologies, such as hypertension, diabetes and hypercholesterolemia on cognitive functioning. Along with population aging, cardiovascular disease remains a critical challenge for public health, in terms of increasing needs and demands for chronic care and sustained prevention.

New holistic approaches are therefore needed to fight efficiently against CVD and their burden on global health. These measures include the development of new definitions and concepts, the investigation of *emerging* (non-classical) cardio-metabolic risk factors, and the epidemiological consideration of associations between CVD risk factors and cognitive and physical functioning, with the ultimate aim to provide appropriate prevention of CVD, and to promote healthy ageing. For example, ranking individuals according to their cardio-metabolic risk status may offer important clues about CVD management, and help to develop evidence-based prevention strategies.

To achieve these objectives, we shall construct a follow-up project, collecting data based on the already constructed national database (ORISCAV-LUX survey); the first nationwide large scale, cross-sectional survey ever carried out in Luxembourg, between October 2007 and January 2009, on a representative sample of 1432 subjects, aged 18-69 years.

The longitudinal dataset will not merely aim to monitor the evolution of major *traditional* cardiovascular risk factors, but also to answer several research questions, as regards the potential role of *emerging* cardio-metabolic risk factors (and risk markers), namely, arterial stiffness, inflammation, and vascular adhesion molecules. Furthermore, a focus on physical and cognitive functioning, environmental pollution and genetic predisposition will be emphasized in specific research questions.

In a coordinated approach, this multidisciplinary research project brings together researchers from diverse disciplines (cardiology, endocrinology, cognition, nutrition, toxicology, body composition, epidemiology and public health). Consequently, it will help to fuel synergetic public

health research inside the L.I.H, stimulate new promising directions, and reinforce international collaborative research networks.

Publication of the research results, in prestigious peer-reviewed journals, will no doubt contribute to the advancement of the current international scientific knowledge and increase the L.I.H. visibility.

1.2. Relevance with the performance contract and the implementation plan of the research unit

Worldwide, longitudinal studies play a key role in the progress of epidemiology, clinical research, and therapeutic/intervention evaluation. Reason for which, almost all outstanding public health research institutions, or Universities embed longitudinal data collection in their research strategy.

In our L.I.H. context, particularly Department of Public Health, the ORISCAV-LUX study was the first nationwide epidemiological and nutritional survey ever carried out in Luxembourg, between the periods of November 2007 to January 2009. Its ultimate aims were to inform prevention and research.

From a public health and epidemiological standpoint, it is important to follow-up this baseline cohort of participants, to monitor the cardiovascular health status of the population, as well as to examine many empirical research questions related to recording of incident events, measurement of individual variation in outcomes, and social change or stability.

The relevance of the second wave of the ORISCAV-LUX can be summarized as follows:

1. To underpin and develop the embryo of the Luxembourg cohort project, in that it provides a 7-year follow-up to the national cohort by considering the ORISCAV-LUX as a pilot study and as the first starting point (baseline).
2. To confederate in-house researchers from different disciplines (public health, epidemiology, cardiology, endocrinology, nutrition, body composition and toxicology) to work together on the constituted dataset, each according to his/her research hypothesis and expertise.
3. To enhance a more integrated and synergistic way of working, inside the Dept. of Public Health, with other researchers at L.I.H. and with international experts in different fields

such as diabetes, cardiovascular diseases, obesity, environmental risks, and ageing-related diseases

4. The ORISCAV-LUX 2 project constitutes a valuable data bank that allows to generate further independent research projects, with the potential support of FNR's grants. These sub-projects will be discussed later in more details.
5. The scientific outputs and peer-reviewed publications will increase the national and international visibility of Luxembourg in the field of cardio-metabolic research, nutrition, body composition, toxicology and aging research.
6. This Follow-up project "ORISCAV-LUX 2" will contribute to reinforce the performance of the L.I.H. and in particular the CES team in the following ways:

    - Increase the number of Master, Doctoral and Post-doctoral students (training and job opportunities).
    - Increase the chance of the CES to submit competitive and contractual projects, for example, FNR-granted projects.
    - Increase the number of national and international peer-reviewed publications in more prestigious journals with high impact factors.
    - Promote inter- and intra-institutional collaboration with the L.I.H., for example (Laboratory of Analytical Human Bio monitoring, Competence Center of Methodology and Statistics, Sports Medicine Research Laboratory, Laboratory of Cardiovascular Research).
    - Maintain the current international collaborations with nominated research institutions (University of South Carolina, USA; University of Maine, Orono, USA; University of South Australia, Adelaide; University of Liege, Belgium, University College of London, UK, and with the Erasmus University Medical Center Rotterdam, the Netherlands).

2. DETAILED DESCRIPTION OF THE PROJECT

2.1. Scientific background

2.1.1. Global burden of cardiovascular disease

Atherosclerotic vascular disease, which encompasses coronary heart disease, cerebrovascular disease, and peripheral arterial disease, is responsible for the majority of cases of cardiovascular disease (CVD)(1). Today around 60% of worldwide deaths and 43% of the global burden of diseases are attributed to coronary heart disease, stroke and type 2 diabetes mellitus (2). These diseases are predicted to account for 73% of global deaths and 60% of the global burden by year 2020 (3). In modern industrialized countries, CVD is the leading cause of hospitalization, disability and death. Despite major advances in prevention, diagnosis and treatment measures, CVD is still the main cause of mortality in Europe. It accounts for over 4 million deaths yearly, i.e., nearly half (49%) of all European mortality burden, but with striking geographical variations.

Our understanding of the pathophysiology of atherosclerotic process and its consequence has been progressing over time, thanks to large prospective cohort studies such as the Framingham Heart Study and the Seven Countries Study. Nowadays, it is well-established that CVD is a multifactorial disorder resulting from a complex interaction of a number of genetic and environmental risk factors, including potentially modifiable lifestyle behaviors(4). **Figure 1** displays the cardiovascular risk factors in relation to the natural history of cardiovascular disease and different levels of prevention.

2.1.2. Traditional cardio-vascular risk factors

"Traditional risk factors", such as cigarette smoking, hypertension, unfavorable lipid profile, obesity and diabetes would explain as much as 85% of the world's burden of atherosclerosis(5), besides unhealthy diet and physical inactivity(6). There is strong evidence that elevated blood pressure, tobacco consumption, hyperglycaemia, overweight/obesity and high cholesterol are not only associated with each other(7), (8), (9) but also with cardiovascular morbidity and mortality(10), (11), (12), (13), (14), (15), (16), (17), (18). At global level, these factors are responsible, respectively, of 12.8%, 8.7%, 5.8%, 4.8% and 4.5% of the total mortality, as well as of 2.3 to 3.7% of the DALY's (Disability-Adjusted Life Year) (19-21).

Current guidelines suggest that lifestyle modifications based on avoiding smoking, taking regular physical exercise, and improving control of hypertension could be the most effective intervention for CVD prevention at the population level(4), (22) .

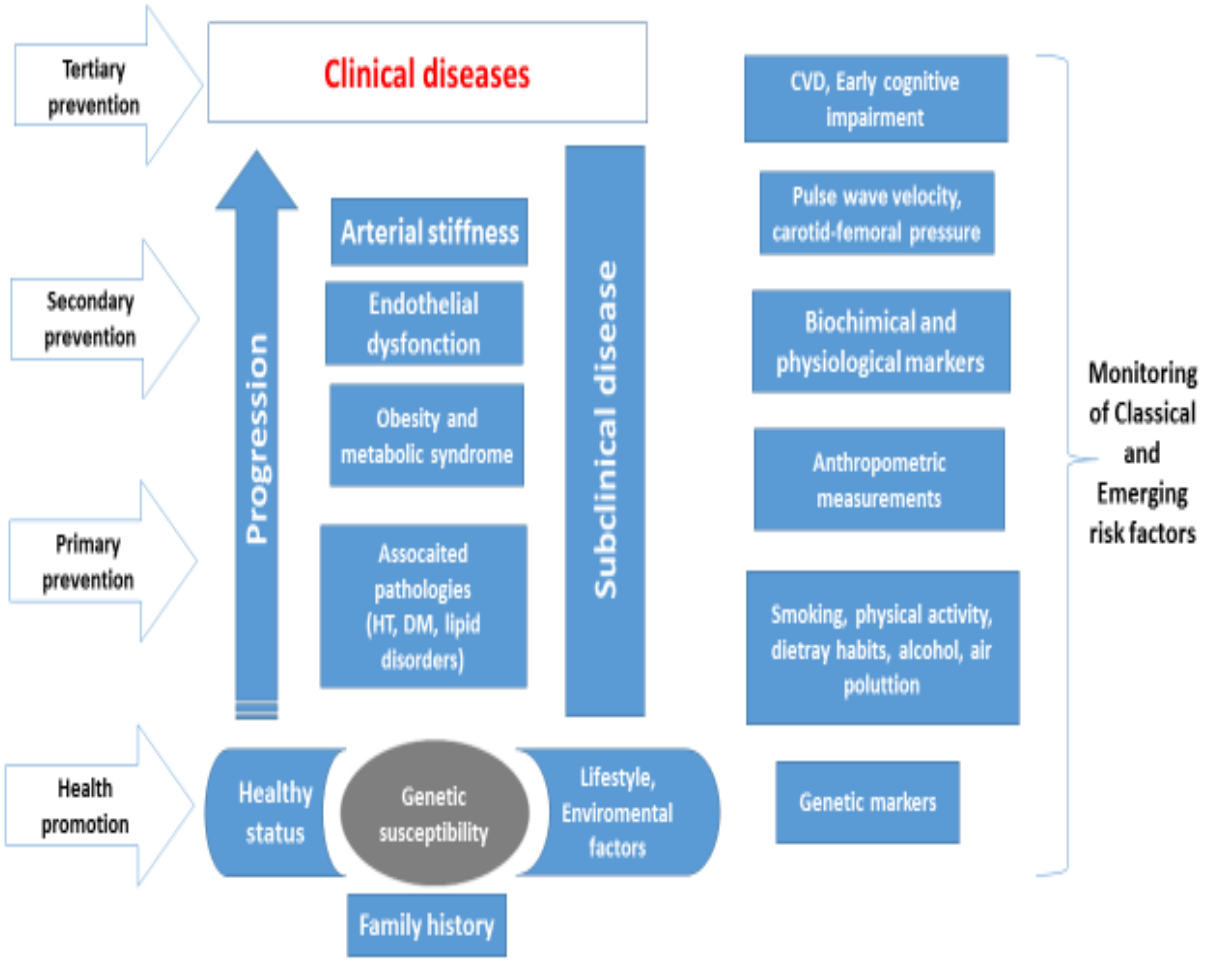


Figure 1 Natural history of diseases and related classical and emerging risk factors, in correspondence with different levels of prevention

2.1.3. Emerging cardio-metabolic risk factors and biomarkers

Besides the "*traditional*" cardiovascular risk factors, a number of other cardio-metabolic markers, so called *"emerging risk factors*", have also been proposed to help identify high-risk individuals(23), (24). These risk factors include, but are not limited to, pro-inflammatory factors(25), sleep deprivation(26), environmental pollutants(27), as well as endothelial dysfunction and micro albuminuria (28). These emerging factors have been proposed as significant predictors of atherosclerosis and its complications in a number of cohort studies, however, replication of these findings and their optimal use in risk stratification remains to be determined.

2.1.4. Arterial stiffness

As age increases, the walls of the aorta and the large elastic arteries progressively harden due to senile degenerative phenomena, represented by the loss of elasticity and the progressive diffusion of atherosclerotic lesions(29). These changes lead to reduce the capacity of the arterial wall to distend during the systole with a consequent increase in both systolic and pulse blood pressure. Although the association of age with various arterial stiffness parameters has been described extensively in the literature, little is known about the clinical significance of arterial stiffness assessment in young subjects.

The central blood pressure has recently achieved much attention for the assessment of cardiovascular risk, notably following the development of a variety of non-invasive methods to evaluate central blood pressure and arterial stiffness(30). Multiple epidemiologic studies have correlated markers of arterial stiffness such as pulse-wave velocity (PWV), augmentation index (AI) and pulse pressure (PP) with risk for the development of fatal and nonfatal cardiovascular events(30), and with mortality in hypertensive patients(31).

In addition, arterial stiffness has been classified as an *emerging* risk factor that provides prognostic information beyond standard stratification strategies, which involve hypertension, diabetes, obesity, dyslipidemia and smoking(30). Given the association between arterial stiffness and cardiovascular outcomes, the possibility of using arterial stiffness, as a treatment target, is of major interest(32).

There are well-recognized associations between *traditional* cardiovascular risk factors and measures of arterial stiffness. Interestingly the adverse consequences of such factors on arterial stiffness, appear to be reversible, as demonstrated in hypertensive smokers(33). Rapid weight gain with visceral fat accumulation appears to be in part associated with the development of arterial stiffening(34). Arterial stiffness appears to be affected by the presence of increasing numbers of components of the metabolic syndrome (35), although the mechanisms remain unclear(32). This pathophysiological and epidemiological evidence has important clinical and prognostic implications in CVD prevention, and particularly in blood pressure management, as acknowledged by the European Society of Hypertension guidelines(36).

Therefore, further research is required to better understand the mechanisms underlying arterial stiffness, its determinants and its potential application in routine clinical care. Considering the novelty of this research area, arterial stiffness and its consequences represent a great challenge of the twenty-first century for affluent countries, and “de-stiffening” is considered to be the goal of the next decades (37).

2.1.5. Healthy / Unhealthy obesity

Paradoxically, recent investigations found that overweight/obesity may favorably impact cardiovascular health. These findings are in particular shown in the clinical cardiology research where overweight/obese people had the best prognosis by surviving a heart attack and/or a heart failure (38). Indeed, recent studies have shown that the relationship between body weight and the incidence/prevalence of CVD is more complex than a simple positive, direct association.

M*etabolically healthy obese* (MHO) individuals, characterized by 1 or 2 cardio-metabolic abnormalities, that include insulin resistance (39-43); hyperglycemia (41-45); dyslipidaemia (41-45); hypertension (41-45); inflammation (43), and/or high waist circumference (39, 43), may show a healthy metabolic profile despite of having a high fat mass (46-48).

In addition, beyond the MHO and the *metabolically unhealthy obese* (MUO) subjects [BMI >= 30], others subgroups have been identified within the normal weight [18.5 = < BMI < 25] and the overweight [25 = < BMI < 30] populations: The *Metabolically Unhealthy Normal Weight* (MUNW) and the *Metabolically Unhealthy Overweight* (MUOV) subjects who display the same cardio-metabolic disorders usually attributed to the MUO; by opposition to the *Metabolically Healthy Normal Weight* (MHNW) and the *Metabolically Healthy Overweight* (MHOV) (43).

A significantly increased arterial stiffness and carotid atherosclerosis were in particular observed in the MUNW compared to MHO and MHNW (49). Independently of the weight status, the metabolically healthy phenotypes might also display a favourable postprandial triglyceride response, compared with the metabolically unhealthy phenotypes (50).

This topic is crucial, especially because the prevalence of the metabolically healthy phenotypes may reach 40% in obese adults, and 50% in overweight adults (43, 51). Unhealthy metabolism might also affect more than 20% of normal-weight adults (43). Nevertheless, because of the absence of a standardized definition, the comparability of the different phenotypes remains difficult. In addition, only limited data concerning the epidemiological and clinical detection of the metabolically healthy/unhealthy weight status are available. Moreover, wrong data interpretation might happen in national statistics and research studies in case of a simultaneous inclusion of both metabolically healthy and unhealthy subject's data. Energy-restricted diet seems also to damage metabolic health in MHO while this therapeutic approach is supposed to improve the glucose / insulin abnormalities in particular (47). Prevention actions and medical care for these phenotypes should be hence adapted.

Determinants of Healthy / Unhealthy metabolic phenotypes

Recent cross-sectional studies tried to define the underlying mechanisms of the *healthy / unhealthy metabolic-weight* phenotypes. Sugar-sweetened beverages were significantly associated to metabolic abnormalities, independently of weight status (52). Physical activity, but not screen time, was significantly associated to the metabolically healthy obese individuals, with regard to the metabolically unhealthy obese (45). The negative impact of aging, low education, moderate alcohol consumption, low physical activity and large waist circumference on the development of cardio-metabolic abnormalities were highlighted in normal weight individuals (43, 53). Metabolically unhealthy obesity was positively associated with persistent organic pollutants (POPs) (54). This finding is of high importance given that POPs were highly associated with visceral adipose tissue (VAT), an extremely relevant and independent cardio-metabolic risk factor (55) . Kim *et al.*, 2014, for example, showed that pesticides and polychlorinated biphenyls (PCBs) were about 5-10 times higher in VAT than in subcutaneous adipose tissue (SAT)(56). Dirinck et al. 2014 observed a positive relationship between persistent organic pollutants and VAT/SAT ratio.

Literature indicates that specific aspects of body composition increase the occurrence of CVD. These include increased visceral adipose tissue rather than global adiposity (47, 57);

decreased subcutaneous adipose tissue (SAT) in the lower limbs (58); decreased lean/muscle mass(59). Peppa *et al.* (2013) highlighted the roles of the central and peripheral adiposity [trunk-to-legs and abdominal-to-gluteofemoral fat ratios] in postmenopausal women (60). Camhi and Katzmarzyk (2014) have shown that a low trunk adipose tissue, VAT, abdominal SAT, total abdominal tissue (TAT) and lean mass (61) were independent determinants of the metabolically healthy phenotype in obese men. However, Kim *et al.* (2013) underlined an inverse relationship between metabolically healthy status and muscle/lean mass (62).

Further investigations remain necessary, concerning the relationship between body composition (total fat mass, visceral fat, muscle mass) and metabolically healthy/unhealthy phenotypes, and not only obese phenotype. The prospective association with food habits, pollutants and sedentarily behaviour analysis, in particular in longitudinal studies needs further investigation.

2.1.6. Environmental exposure to organic pollutants – biomonitoring and study of exposure-associated effects

Background

Although genetic factors have long been prominent objects of study for explaining chronic diseases, an increasing amount of studies suggest a critical role of environmental exposure. Actually, several authors suggest that a large part of chronic affections such as cancer or cardiovascular disease cannot be explained by the genes, and therefore, point out environmental factors (63-66).

Studying the role of the environment in the etiology of disease is a complex task facing several difficulties, starting with the definition of the "environment" itself. In a broad sense, "environment" includes all the non-genetics factors (*e.g.* diet, behavior, lifestyle, infectious agents, etc). A more focused and easily admitted definition is however limited to exposure to pollutants through air, water, soil and diet, but also to physical agents (radiations) and includes occupational exposure (67, 68). Among the different factors included in the latter restricted definition of "environmental factors" are organic pollutants (OP). These compounds may be produced on purpose for specific applications (*e.g*. pesticides, flame retardants), as secondary compounds or partial degradation by-products of aging materials (*e.g*. phtalates, phenols, PCBs) or as wastes from industrial processes or other human activities (*e.g*. polycyclic aromatic hydrocarbons, dioxins). Due to their continuous release into the different environmental compartments, these compounds have contaminated most of the human surroundings,

resulting in the chronic exposure of people mainly through diet, water and air. Exposure to such chemicals may be increased for some specific categories (occupationally exposed, smokers, inhabitants of contaminated areas) but somehow concerns the entire population.

An area of increasing interest is the contribution of organic pollutants exposure to metabolic disease such as insulin resistance, obesity, diabetes and associated complications (69-74). Such associations were actually reported within several experimental studies on animal models and cell cultures (75-78). For instance, Ruzzin et al demonstrated that rats exposed to a 28 days-consumption of salmon oil containing persistent organic pollutants (organochlorine pesticides, dioxins and polychlorobiphenyls) developed insulin resistance, abdominal obesity and hepatosteatosis, whereas this was not observed in rats submitted to similar diet without POPs. The contribution of POPs to insulin resistance was confirmed in cultured adipocytes where pollutants, especially organochlorines, led to robust inhibition of insulin action and induced down-regulation of insulin-induced gene-1 and Lpin1 which are master regulators of lipid homeostasis (75). Similarly, Arzuaga et al studied the influence of exposure to PCB77 on mice submitted to diet enriched with high-linoleic acid oils. Microarray analysis revealed down regulation of fatty acid metabolism, triacylglycerol synthesis and cholesterol catabolism only for mice exposed to PCB77(76). Similar effects were also demonstrated for non-persistent contaminants such as bisphenol A. In a recent study, Marmugi *et al* demonstrated that after 8-months exposure to BPA through drinking water at doses below or equivalent to the current no observed adverse effect level (NOAEL), male mice presented significant impact on their glycemia, glucose tolerance and cholesterolemia (79).

The challenge of exposure assessment

The findings described above are consistent with epidemiological studies which reported association between exposure to organic pollutants with cardiovascular disease (CVD) or with a series of biological disturbances such as diabetes, obesity, dyslipidemia or insulin resistance, known to subsequently lead to CVD(80-84). Epidemiological evidence however remains controversial because most studies are based on cross-sectional design and also because of the uncertainty associated with the exposure assessment. Indeed, mainly due to logistic and financial constraints, exposure assessment most often relies on a single biological samples per individual, mainly blood and urine, the latter being generally preferred due to its non-invasive sampling (85). Nevertheless, for most compounds, the rapid elimination from urine after exposure stops results in short window of detection (the period of time before sampling measured concentration is representative of) and in high variability in chemicals urinary

concentration (86, 87). The variability associated with chemical concentration in biological fluids, especially for short half-life chemicals, increases the risk of misclassification of individuals with regard to their exposure level and results in dramatic loss in statistical power in the study of associated adverse health effects.

In order to tackle the limitations associated with conventional matrices, novel approaches based on alternative biological matrices such as hair are being developed (88). Several works thus reported the possibility to detect in hair organic pollutants from different chemical classes, reflecting individuals' environmental or occupation exposure. Along with the easiness of sampling and storage of hair samples, the main advantage associated with this matrix lies in the possibility to reach extended windows of detection that may represent up to several months, depending on specimen length. Contrary to biological fluids such as urine and blood, the concentration of chemicals detected in hair is not influenced by short-term variations in the exposure and corresponds to the individuals' average level of exposure, which is the most relevant information for investigating possible relationships with biological effects. Although the lack of sufficiently sensitive analytical methods has for a long time limited the possibility to properly monitor environmental exposure, considerable efforts have been concentrated on this aspect in the past few years. For instance, recent works conducted in the laboratory of analytical human biomonitoring (LAHB, L.I.H) combining solid phase microextraction (SPME) with gas chromatography tandem mass spectrometry (GC-MS/MS) for the detection of pesticides in hair allowed reaching sensitivity levels that stand between 1000 and 10,000 times below those obtained ten years ago (89). In addition to "classical" compounds most frequently investigated in the different studies (*e.g.* organochlorines), new methods have been developed for the analysis of compounds that had not been analyzed in this matrix so far. For example, a method developed in the LAHB demonstrated for the first time the possibility to assess exposure to polycyclic aromatic hydrocarbons by the determination of their mono-hydroxy metabolites in hair (90, 91). In parallel, focus has been set on the development of multi-residue analysis, with a view to increasing the number of different chemicals simultaneously detected in a single specimen (89, 92, 93). These advances meet the recent findings that point out the biological effects associated with cumulative exposure to low levels of several different chemicals (94, 95). In addition to the extended windows of detection which make hair the most suitable biological matrix for investigating chronic exposure, the analytical performances reached nowadays clearly bring to the fore its relevance within the context of epidemiological studies aiming at assessing the impact of environmental exposure on pathophysiological effects onset.

Exposure-associated adverse effects in ORISCAV-LUX

The relevance of hair analysis for the assessment of human exposure to organic pollutants was highlighted through several recent results from the Laboratory of Analytical Human Biomonitoring of LIH (89, 91, 92). Although some previous studies already conducted population exposure assessments, results demonstrated that each population have its own specificities regarding the pollutants people are exposed to, and hence the associated risks. As no such study has been carried out in Luxembourg yet, ORISCAV-LUX 1s the very first opportunity to provide exposure data on the Luxembourg population. As the previous studies in the field were based on the analysis of urine or blood, which were shown to be less reliable than hair for the biomonitoring of most compounds, the specificity of using hair for exposure assessment is a significant novelty of this project. Although a few studies were already conducted with hair analysis, most of them actually consisted in feasibility studies with limited number of chemicals tested in a limited number of individuals, or concerned specific populations mainly submitted to occupational exposure. The present study will therefore be the first one to involve such a large number of people representative of general population. The wideness of the list of pollutants simultaneously analysed here, including “novel” contaminants such as bisphenol and phthalates, will moreover provide a level of cumulative exposure assessment that had never been reached so far. The ORISCAV-LUX study is also the first one to investigate associations between biological adverse effects and exposure assessed by means of hair analysis.

The analysis of biomarkers of tobacco smoke in hair can be considered the most powerful tool for assessing both active smoking and secondhand smoke exposure in non-smokers on the mid-term. It provides objective value of the consumption magnitude in smokers, avoiding bias associated with self-declaration, and enables to document passive smoking in people that would categorize themselves as non-smokers. The first results obtained by LAHB for the Luxembourg population reported a significant level of second hand smoke exposure before smoking ban (91). Although some studies provided assessments of the effects of smoking ban on exposure and tobacco consumption, most were limited to specific contexts (catering staff, pregnant women…) and based on indirect evaluation. The follow up of the ORISCAV-LUX population will therefore provide the first assessment of the effect of smoking ban on exposure to secondhand smoke using objective and reliable means such as hair analysis, in a cohort representative of the country’s general population.

2.1.7. Aging, CVD risk and cognitive impairment

Similar to other European countries, Luxembourg is an aging society. Intact cognitive function is a critical dimension of life quality, as cognitive difficulties can be disruptive to individuals' well-being and to their everyday functioning. Recent literature has shown that cognitive decline becomes more common with ageing and interferes with daily functioning and well-being.

It has been shown that across the life span, several factors can negatively impact cognitive function and contribute to accelerate age-related cognitive changes (96). Factors that influence the development of chronic diseases, such as poor lifestyle habits, biological risk factors, hormones, genetic factors, exposure to environmental toxins, and certain medications, can have an adverse effect on cognitive function. Several CVD risk factors, including high blood pressure, dyslipidaemia, smoking and obesity, have been proposed as important modifiable risk factors for cognitive decline (18), (19). So far, longitudinal studies that explored the association of cardiovascular risk factors with cognitive functioning produced contradictory or inconclusive findings, particularly pertaining the blood pressure (20), (21), (22) serum cholesterol (23), (24) and BMI (25), (26).

In addition, prior to the development of cerebrovascular complications, even early manifestations of CVD, such as hypertension, are associated with diminished cognitive function (97). Despite an overall increase in hypertension-related risk for poor cognitive function, there are striking inter-individual variability in performance within hypertensive groups(97). The interactive effects of hypertension with many socio-demographic (e.g., gender, race/ethnicity, socio-economic status), lifestyle, genetic, and other biological factors are also possible, yet they remain partially explored.

With respect to health-compromising behaviors, several studies have revealed poorer cognitive performance among individuals who smoke tobacco products (98). In addition, several dietary insufficiencies, such as vitamin B6, vitamin B12, thiamine, folate, and zinc, have been related to cognitive difficulties(99). Environmental or occupational exposure to chemicals, such as solvents and lead, exerts direct neurotoxic effects on the brain and is associated with diminished cognitive functioning (100).

New emerging CVD risk factors, in particular arterial stiffness has also been detected as an important predictor of cognitive decline and dementia (101) . Arterial stiffness, as measured by Pulse wave velocity (PWV), is a measure of subclinical CVD risk (102), (103) that progressively

increases with age (104). Arterial stiffness largely contributes to systolic hypertension, the most common form of hypertension among older adults (105), (106). Independent of traditional CVD risk factors, arterial stiffness has been linked to damages in the central pulse pressure (107), resulting in changes in the functioning of the frontal–subcortical regions of the brain which may, in turn, influence cognitive decline (108).

From public health standpoint, *traditional* and *emerging* CVD risk factors, particularly PWV may constitute a useful and noninvasive measure in predicting the risk of cognitive decline among older adults (109), (110). Longitudinal research is required to confirm causality, as interventions to prevent arterial stiffness may be effective in delaying cognitive decline.

2.1.8. Aging and physical functioning

Not all individuals age at the same pace; some of them enter in old age without any disease and totally functional, whereas others just survive from diseases, disability and frailty (111). Frailty is a condition of vulnerability in face to a stressor (112) and can be considered a counterpart of healthy ageing. Frailty is associated with higher risk of mortality, hospitalization, institutionalization, disability, fractures and falls in aged population (113). Therefore, this condition should be diagnosed early and prevented.

Frailty includes several components of biological ageing (114). Physical functioning has been used earlier as a proxy measure of frailty. Physical functioning is present in the beginning of the decline process and is characterized by irreversible changes in the sensory and motor functions. A diminished physical functioning is an early indicator of health decline and risk factor for poor quality of life in elderly people (111).

So far, few studies have focused on physical functioning, showing that its components such as walking speed (115, 116) and grip strength (117) are good predictors of mortality. Both instruments have the advantage of being objective and easy to measure. Besides, there are still questions concerning the possible determinants and mediators of physical functioning in the general population, especially from a longitudinal point of view. There is little evidence about the association between physical activity and the risk of future impaired physical functioning. It is not clear whether physical activity acts as mediator or determinant, to help understand any independent contribution to the global frailty concept.

Prevention of frailty should be a public health priority. With respect to current health policies, this follow-up ORISCAV-LUX 2 project will generate evidence to help improve guidelines and

recommendations for general practitioners and public health professionals about how to detect frailty and avoid its potential risk factors.

2.1.9. Genetic predisposition

Rare single genetic variants that give a high risk of disease have been successfully identified in the past using linkage analysis. Cystic fibrosis or Huntington disease are good examples among many others of such monogenic or Mendelian diseases(118), (119). However in the case of complex diseases like CVD, diabetes or conditions like obesity with multiple gene variants making a small contribution to disease heritability and with environmental or behavioural risk factors playing an important role the situation becomes more complex.

Exploring at the genome scale the association between gene variants and complex disease susceptibility was made possible only after some major recent advances. The successful completion of HAPMAP project that provided in 2005 and 2007 a map of human genetic variations and linkage disequilibrium(120), (121) and the availability of high-density Single Nucleotide Polymorphisms (SNP) genotyping made possible the genome wide association studies (GWAS) (122).

In the beginning a genetic predisposition for a disease was thought to be a mutation that will alter the structure of a protein coded by the mutated gene. The final result could be a less functional protein that will increase the risk for a particular disease. It is therefore expected to find the mutation more often among people with the disease than among healthy controls. However only 1% of our DNA is responsible for coding proteins and a great number of GWAS also reported SNP-s in the non-protein coding regions of the genome to be found more frequently among cases than healthy controls(123).

In a case-control perspective ORISCAV-LUX 2 project presents a unique opportunity to collect data on health status, traditional and emerging risk factors and on genetic predisposition from a representative sample of the resident population. The risk profile of participants without a specific disease or condition will be compared with that of cases with a disease or condition selected among the hospital diagnosed patients. In a longitudinal perspective ORISCAV-LUX 2 is the embryo of a larger cohort that will have the potentials to study gene-environment interactions for a series of diseases and conditions in the Luxembourg's population. The future benefits for the patients, could range from personalised preventive medicine, to treatments tailored to patient's genetic make-up.

2.2. Our national context

The Grand-Duchy of Luxembourg is a small country in the heart of Europe landlocked by Belgium, France and Germany, with a population of 549,700 inhabitants (official estimate, STATEC, 2014) over an area of about 2600 km^2. About 45% of the population subjects are well-integrated foreign residents from over 150 different nationalities. In Luxembourg, death from circulatory system diseases accounted for about one-third of total mortality, with a stable trend since last decade(124). This outcome made cardiovascular health one of the top priorities of Luxembourg decision-makers and healthcare authorities.

Face to the elevated CVD mortality during the last decade, the Ministry of Health has long been involved in planning and organizing prevention programs and health promotion campaigns to endorse healthy lifestyles. However, relevant evidence-based observational data were necessary to monitor public health indicators and to control efficiently risk factors.

2.3. ORISCAV-LUX 1 (November 2007- January 2009)

In 2007, the Ministry of Health and the Ministry of Research supported the L.I.H (former CRP-Santé) by providing a two-year funding for the first epidemiological "Observation of Cardiovascular Risk Factors in Luxembourg" (ORISCAV-LUX) study(125), (126). It was designed as a nationwide population-based cardiovascular survey, aimed to establish baseline information on the prevalence of potentially modifiable and preventable CVD risk factors, including obesity, hypertension, diabetes mellitus, lipid disorder, current smoking, physical activity and dietary habits among the general adult population of Luxembourg.

2.4. Our relevant previous work

Epidemiological status of potentially modifiable CVD risk factors in Luxembourg

The findings from the ORISCAV-LUX study revealed a high prevalence of potentially modifiable and treatable cardiovascular risk factors among apparently healthy subjects(125), (127). The most predominant cardiovascular risk factors were dyslipidemia (69.9%), hypertension (34.5%), smoking (22.3%), obesity (20.9%), and diabetes (4.4%). Significant gender and age-specific differences were seen not only for single, but for combined risk factors. Only 14.7% of men and 23.1% of women were free of any cardiovascular risk factor (125). Alarmingly, the younger age group (18–29 years) already presented a multiple risk profile (125), (128).

CVD risk in Portuguese immigrants versus Luxembourgers

Luxembourg country is a multicultural society; about 43.2% of the people are foreign residents from over 150 different nationalities, among those, the Portuguese representing the major immigrant community (36.7%). Our findings indicated that the distribution of cardiovascular risk factors was almost similar in both Luxembourgish and Portuguese populations, with no significant difference as regards hypertension, lipid disorders, diabetes, smoking status and physical inactivity, except for overweight/obesity, which was more common in Portuguese immigrants than Luxembourgers (129).

Population awareness of CVD risk factors

Strikingly, our recent findings showed that 85%, 60%, and 32%, were respectively unaware of their dyslipidemia, hypertension and diabetes (128). These pathologies commonly co-exist and constitute the most common risk factors for coronary heart disease (CHD). Subjects who suffer from these pathologies are often unaware that they are afflicted until they experience debilitating complications. Patients with silent hypertension associated with dyslipidemia and uncontrolled diabetes are often susceptible for premature myocardial infarction and hemorrhagic stroke. The asymptomatic character of these treatable and preventable risk factors contributes to increasing the incidence of cerebrovascular accidents and of sudden death. The estimated thousands of people, particularly of old age group (50–69 years) with these treatable and preventable cardiovascular risk factors, but unaware of their pathologies are worrying. More worrisome is that approximately two-thirds of treated cases of diabetes, hypertension and dyslipidemia were not correctly controlled according to clinical guidelines (128).

Epidemiology of the metabolic syndrome and its determinants in Luxembourg

In Luxembourg, more than one fourth of the resident population (28%) were affected by the metabolic syndrome (MetS); this prevalence increased with age and was significantly higher in men than in women(127). The MetS represents a clustering of central adiposity, dyslipidemia, hyperglycemia and elevated blood pressure. The significance of this worldwide emerging cardio-metabolic disorder is related to its association with an increased risk of developing type 2 diabetes and atherosclerotic CVD. Although its pathogenesis is still incompletely understood, the interaction of genetic and environmental factors such as obesity, dietary and sedentary lifestyle, is known to contribute to its development(130), (131).

Our research findings indicated that several dietary, socio-economic and behavioral factors explain the disparity observed in Luxembourg population with respect to the MetS (132). Beyond the appropriate medical treatment for those already affected, the public health approach of primary prevention is considered to be the most cost-effective, affordable and sustainable course of action to cope with this global epidemic(133). Currently, healthy eating promotion messages occupy a prominent position in prevention activities.

Compliance to national dietary guidelines

In 2006, the national public health authorities in Luxembourg launched an intensive campaign with the aim of providing the general population with food- and nutrient-related guidelines. The achievement of appropriate dietary habits are a major public health concern because inadequate diet constitutes a potential health threat. Therefore, representative data of population dietary intake from the ORISCAV-LUX survey 2007–8, were used to investigate the population compliance with current national dietary recommendations, emphasizing both the adherence to food recommendations that reflects appropriate food selection, and the adherence to nutrient recommendations that reflects good-quality dietary intake. Our findings demonstrated that a substantial proportion of the population of adults residing in Luxembourg exhibits a poor to moderate compliance, implying that dietary habits of adults ought to be improved(134). In addition, the segment of the population with poor compliance was represented by young adults, deprived subjects, smokers and those unaware of the importance of a balanced diet to maintain good health. The magnitude of the gap between current dietary intakes and the national recommendations calls for a continued effort to increase the population awareness of the importance of a healthy and balanced diet.

Focus on Obesity-Nutrition Research

Obesity is a rapidly worsening public health problem associated with a variety of co-morbidities including type 2 diabetes, hypertension, stroke and CVD(133). Obesity is impacting heavily upon healthcare systems around the world, including Luxembourg, where 21 % of the population is obese (125). Obesity occurs in the context of a variety of interrelated demographic, socio-economic and lifestyle factors; nutrition is coming to the fore as a major modifiable determinant. Proper nutrition offers one of the most effective and low-cost strategies to decrease the burden of many diseases and their associated risk factors, such as obesity.

- **Ready-made meals and obesity**

Over recent years, there have been major alterations in dietary habits, with growing transition from home-made to eaten-out foods and pre-prepared meals (including 'heat-and-eat' foods, fast foods or takeaways). This shift has been concomitant with the current obesity epidemic, raising the possibility that these two trends are causally related. Our recent original findings from the ORISCAV-LUX study has recorded about 97 % of the participants reported daily consumption of ready-made meals in different quantities. The intake was highly prevalent in men living alone and varied according to education level. Ready-made meal consumption provided >7 % of total daily energy. The fractions of macro- and micronutrients derived from daily consumption of ready-made meals varied from 10 % for total cholesterol to 0·65 % for total fiber. Increased consumption of ready-made meals was independently associated with abdominal obesity, a major indicator of body fat deposition(135). The observed difference appeared to be largely independent of other potentially confounding lifestyle and dietary factors, including the intakes of alcoholic and non-alcoholic beverages, fruits and vegetables, added fats, grains, meats and dairy products. In addition, on controlling for age, sex, socio-economic status and lifestyle factors, daily consumption of ready-made meals was associated with higher energy intake and with poor compliance with national nutritional recommendations, and hence it could plausibly increase the risk of central obesity and fat deposition.

- **Dairy food intake and obesity**

Our cross-sectional analyses showed that higher total dairy food intake was significantly associated with a lowered prevalence of global and abdominal obesity, by up to 50%. Participants in the highest tertile of whole-fat dairy intakes (milk, cheese, yogurt) had significantly lower odds for being obese (global obesity: OR, 0.45; 95% CI, 0.29-0.72; $P < .01$; abdominal obesity: OR, 0.35; 95% CI, 0.23-0.54; $P < .001$), compared with those in the lowest intake tertile, after full adjustment for demographic, lifestyle, dietary, and cardiovascular risk factor variables. This relationship was particularly evident for whole-fat dairy products, namely, milk, yogurt, and cheese(136). Our results suggest that increasing consumption of dairy foods may have the potential to lower the prevalence of global and abdominal obesity. These findings are consistent with those from a recent prospective 12-year follow-up study carried out in Sweden, which provides evidence for the beneficial effects of whole-fat dairy food consumption (137).

Additionally, total dairy food intake was positively associated with cardiovascular health of the adult Luxembourg people, as indicated by a higher cardiovascular health score (CHS)(138). Specifically, higher intakes of whole fat milk, total yogurt, and total cheese were independently

associated with better cardiovascular health. Whole fat dairy food intake was strongly correlated with other positive health behaviors including non-smoking, better dietary intakes, and normal BMI. Future longitudinal studies and intervention trials are needed to substantiate these findings.

- **Animal protein sources and obesity**

Furthermore, our findings suggest that protein derived from animal sources, in particular from meat, fish and shellfish, may be associated with increased risk of both global (OR=1·15; 95 % CI 1·09, 1·22) and abdominal obesity (OR=1·12; 95 % CI 1·07, 1·18) among adults in Luxembourg, independently of gender, age, educational level, smoking status, physical activity and intakes of fibre, fat and carbohydrates. Protein derived from eggs or milk products was unrelated to global or abdominal obesity(139).

These findings suggest that lower animal protein intakes may be important for maintenance of healthy body weight. Despite our intensive research to examine the diet-CVD risk associations, further prospective follow-up investigations are required to substantiate our previous findings and to confirm potential causality.

Diet quality and cardio-metabolic biomarkers

The scientific community has become increasingly interested in the overall quality of diets rather than in single food-based or single nutrient-based approaches to examine diet–disease relationships.

Recently, there has been an influx of research attention regarding the anti-inflammatory role that diet has in chronic and metabolic diseases. A literature-based dietary inflammatory index (DII) to characterize the inflammation-modulating capacity of individuals' diets has even been developed and validated in an American population. In collaboration with the team from South Carolina, USA, who developed the DII, we tested its inflammatory capacity in our European population.

In our population, the DII ranged from a minimum of -4.02 (most anti-inflammatory) to a maximum of 4.00 points, with a mean value of -0.41. Participants with higher DII score were significantly younger and had lower body mass index, waist circumferences, and systolic blood pressure levels. Other cardiovascular biomarkers including diastolic blood pressure, CRP, lipids, and glycemic biomarkers did not vary significantly across DII tertiles. Participants with pro-inflammatory DII scores (>1) had increased adjusted odds (odds ratio, 1.46; 95% confidence interval, 1.00-2.13) of having a low high-density lipoprotein cholesterol, compared with those with anti-inflammatory scores. There were no significant relationships between high-sensitivity CRP and the DII(140). This study, which tested the inflammatory capacity of the DII outside the United States, did not detect a significant independent relationship with cardio-

metabolic biomarkers, by using Food Frequency Questionnaire-collected data. These results are informative and representative of a relevant step in directing future research for nutrition and diet quality.

Cross-dietary indices comparison

Beside the DII, there are a plethora of indices used to measure diet quality. However, there still exist questions as to which of these can best predict health outcomes. Therefore, we focused our further research on cross-comparison of diet quality indices for predicting chronic disease risk. In this study, we compared the ability of five diet quality indices, namely the Recommendation Compliance Index (RCI), Diet Quality Index-International (DQI-I), Dietary Approaches to Stop Hypertension (DASH), Mediterranean Diet Score (MDS), and DII, to detect changes in chronic disease risk biomarkers. Among these five selected diet quality indices, the MDS exhibited the best ability to detect changes in numerous risk markers and was significantly associated with lower levels of LDL-cholesterol, apo B, diastolic blood pressure, renal function indicators (creatinine and uric acid) and liver enzymes (serum γ-glutamyl-transpeptidase and glutamate-pyruvate transaminase). Compared with other dietary patterns, higher adherence to the Mediterranean diet is associated with a favourable cardio-metabolic, hepatic and renal risk profile(140). Our findings suggest that diets congruent with current universally accepted guidelines may be insufficient to prevent chronic diseases, and clinicians and public health decision makers should be aware of needs to improve the current dietary guidelines.

Physical activity and cardiovascular health

Physical inactivity is a well-known modifiable risk factor. Our cross-sectional investigations based on the ORISCAV-LUX study have demonstrated that sitting time and time spent viewing television and using a computer, were inversely associated with ideal cardiovascular health, as indexed by seven health factors and behaviors (smoking, BMI, recommended and non-recommended diet, total cholesterol, fasting plasma glucose, and blood pressure). Higher weekday sitting time (including sitting time in front of the television, at a computer, at place of work, and during transportation) was associated with a lower CHS (141). When television and computer time were analyzed separately, television time was inversely associated with the CHS, on both a workday and a day off, regardless of age, gender, education, profession type and income. Those watching television for less than two hours per day had significantly better health scores than those watching television for three or more hours per day. For computer

time, an inverse relationship was observed on days off, suggesting that participants spending more than three hours per day on a computer during their days off from work are more susceptible to having poor cardiovascular health. From a public health standpoint, this finding is important. Reducing sedentary behavior during 'free or leisure time' may be a particularly important message to those who have sedentary or computer-based occupations during the working week. Future studies should explore whether different sedentary behaviors have differential associations with health outcomes, particularly utilizing both self-report and objective measures of sedentary time and energy expenditure(142).

Impact of CVD risk on cognitive function

Results of a large body of research indicate that hypertension is associated with poorer cognitive functioning across multiple domains of performance, and that chronic hypertension predicts cognitive decline over time. In this context, our current research on similar American study (MSLS), in collaboration with the University of Maine, USA, has shown that better cardiovascular health, indexed by a higher number of CVD metrics at ideal levels, is associated with greater cognitive performance. Smoking, physical activity, and diet are important components of cardiovascular health that impact upon cognition(143). Health-enhancing behaviors have been associated with better cognitive functioning.

In addition, we found that higher cognitive performance is prospectively associated with healthy dietary choices in the same MSLS study (manuscript accepted for publication in *Journal of Prevention of Alzheimer's Disease* 2015). There are significant independent relations between higher cognitive performance and greater consumption of vegetables, meat, and fish, and lower consumption of grains. These data suggest that cognition early in life may influence dietary choices later in life. There are many avenues of research to pursue in terms of further characterizing the relation of health and illness to cognitive function. Therefore, it is important to replicate these findings in European settings. The Follow-up ORISCAV-LUX 2 project will permit this opportunity.

From a public health standpoint, it is critical to examine the impact of several chronic comorbidities on cognition. To date, the cognitive consequences of various diseases have typically been studied in isolation. However, comorbidities are extremely common, particularly among older adults. Consider, as one example, that the coexistence of smoking, heavy alcohol consumption, physical inactivity, hypertension, dyslipidaemia, diabetes mellitus, atherosclerosis, cardiac arrhythmias, myocardial infarction, and various medications in an

individual patient is not unusual. As mentioned above, recent research has indicated that the presence of several cardiovascular risk factors (e.g., hypertension, diabetes, and smoking) confers greater risk for poor cognitive performance than any single factor considered in isolation. Considering our multi-risk population, further research in this direction is important, to better understand the factors that predict the cognitive health or decline.

Clinical research on cardiac biomarkers

Recently, the cardiovascular research group at L.I.H. reported significant associations between cardiovascular risk factors and the levels of cardiac biomarkers such as microRNAs (144) and long noncoding RNAs (145), observations that find their relevance in the context of an active area of investigation of circulating RNAs as novel cardiac biomarkers (146).

Findings from the Luxembourg Acute Myocardial Infarction registry indicate that age (hazard ratio, 1.40; 95% CI, 1.26 to 1.56), peak creatine kinase level (hazard ratio, 1.08; 95% CI, 1.02 to 1.14), and diabetes (hazard ratio, 2.13; 95% CI, 1.34 to 3.40) were the only significant predictors of survival (147).

Metabolically healthy / unhealthy weight status

Concerning cardio-metabolic risk stratification, so far, there is no published data and/or debate related to the metabolically healthy/unhealthy obesity, overweight and normal weight issue in Luxembourg. In our preliminary analysis on the ORISCAV-LUX database, we found that among the obese subjects, there were 70 subjects with MHO profile against 255 MUO subjects. Among the overweight subjects, around half (218 subjects) were MHOV compared to 261 MUOV. Likewise for normal weight subjects (296 MHNW compared to 309 MUNW). Further, it is interesting to identify the evolution and determinants of the metabolically healthy/unhealthy weight status phenotypes.

Toxicology findings

The analysis of hair samples collected within ORISCAV-LUX clearly demonstrated the multiple exposure of the volunteers to organic pollutants. Although some chemicals appeared to be common to the three populations under study, regional differences were also highlighted for some compounds between Luxembourg, Wallonia and France. Results analysis also revealed several associations between exposure and some cardiovascular risk factors.

Tobacco smoke consumption (active smoking) or exposure (passive smoking) was initially evaluated within ORISCAV-LUX on the basis of biomarkers concentration in hair samples (nicotine/cotinine) compared to volunteers' self-declaration (148). Preliminary results based on 105 subjects confirmed the suitability of hair analysis to highlight both tobacco smoke and exposure to secondhand smoke in non-smokers. These results also demonstrated that about 1/3 of the non-smokers were exposed to secondhand smoke. The re-examination of the same population by the same means more than one year after law implementation will provide information on the effect of banning smoking in public places on both tobacco smoking and exposure to secondhand smoke.

2.5. Holistic approach of CVD prevention - Key for global Health

CVD prevention is a crucial issue of contemporary global public health. Since decades, it was evidenced that modifications of lifestyle, social and environmental behaviors can induce major changes in the risk of CVD (149), and even modest changes in CVD trends demonstrated a huge public health impact (150).

However, despite remarkable advances in cardiovascular health promotion over the past several decades, CVD remains the leading cause of death worldwide, and in Europe, including Luxembourg, with obesity being an escalating contributor (151). Moreover, rapid population aging, and its health consequences constitute an additional critical public health challenge. Prevention is a key issue in terms of keeping health systems economically feasible and affordable(152). For example, preventing avoidable degenerative diseases is necessary to keep resources available to treat diseases that cannot be prevented, and to improve healthy ageing and societal productivity and well-being at all ages.

Therefore, new knowledge and novel holistic approaches are needed to fight against CVD and their burden on global health. These measures include the development of promising lines of research that focus on newly *emerging* (non-classical) cardio-metabolic risk factors, along with deep consideration of the epidemiological significance of novel hypotheses, related to heart-brain link and gene-environment interaction. These approaches will help to provide better understanding of the CVD prediction, and hence appropriate management of high-risk subjects.

2.6. Intended complementary research and future perspectives

In this follow-up project, our challenge is not merely to monitor the trends of the "*classical*" above-mentioned potentially modifiable and treatable risk factors, but also to monitor and

identify the determinants of the "*emerging"* risk factors, such as inflammation, thrombosis, metabolically healthy/unhealthy weight status, cardiac biomarkers and arterial stiffness.

Accumulating littrature has evidenced that factors related to both the individuls and the enviroment could explain the observed elevated prevalence and poor management of CVD risk and pathologies. Therefore, longitudinal assessment of Luxembourg population's exposure to a list of environmental contaminants will be done. In addition, a focus will be emphasized on the novel research hypothesis, suggesting heart-brain link (i.e., the impact of *traditional* and *emerging* CVD risk factors on cognitive fonctioning).

In sum, the follow-up ORISCAV-LUX 2 study has two major perspectives:

1. **Public health perspective:** to monitor the temporal evolution of potentially modifiable CVD risk factors such as metabolically healthy/unhealthy obesity/overweight/normal weight, hypertension, diabetes, lipid disorders, smoking, and physical inactivity, allowing thus public health authorities and health professionals to develop evidence-based prevention strategies to tackle the real needs of the population.

2. **Research perspective**: these longitudinal data constitute a worthy tool attracting researchers to investigate thoroughly several research questions, **for example:**

    - Investigate new cases (incidence rate) of cardio-metabolic conditions, such as diabetes, hypertension, dyslipidemia, obesity, metabolic syndrome.
    - Investigate new cases (incidence rate) of the metabolically healthy/unhealthy weight status phenotypes (normal weight, overweight and obesity).
    - Describe the body composition of different metabolically healthy/unhealthy phenotypes.
    - Identify the determinants (diet, physical activity, pollutants, body composition) of the metabolically healthy/unhealthy weight status phenotypes.
    - Investigate new cases (incidence rate) of early cognitive impairment and early manifestations of atherosclerosis, such as arterial stiffness.
    - Examine the impact of *traditional and emerging* CVD risk factors on cognitive performance.
    - Investigate the relationship between the metabolically healthy/unhealthy weight status and the cognitive function.

- Investigate the relationship between body composition and cognitive decline.
- Investigate the relationship between the metabolically healthy/unhealthy weight status and arterial stiffness.
- Investigate the relationship between body composition and arterial stiffness.
- Examine the impact of CVD risk factors on the levels and predictive value of traditional and emerging cardiac biomarkers.

- Investigate the association between genetic predisposition and Coronary Heart Disease susceptibility

- Investigate the association between genetic predisposition and susceptibility to other complex chronic diseases.

- Examine the impact of environmental or occupational exposure to pollutants on cognitive performance.
- Assess the prevalence of frailty in the ORISCAV-LUX population.
- Study the combination of subjective measures of physical activity with objective methods and cardiovascular fitness as outcome.
- Investigate the role of physical activity and sedentary behavior in the development of the syndrome of frailty, with physical functioning as a proxy in the general adult population.
- Study the association between physical activity, diabetes and obesity at baseline with physical functioning at the follow-up, and examine different aspects of physical activity (intensity, duration, or type) in their prospective association with physical functioning.
- Investigate the relationship between the metabolically healthy/unhealthy weight status and frailty.
- Investigate the relationship between body composition and frailty.
- Comparison of the information obtained from classical versus “newly used” matrices regarding exposure assessment. This will represent an opportunity to apply new approaches recently developed at LAHB on the bio monitoring of human exposure to the framework of an extended cohort.
- Investigate the bio monitoring of exposure to endocrine disruptor pollutants that were not analyzed in the first step of the study, namely bisphenol-A and congeners, phthalates and other phenols.

- Observe trends in human exposure to “reference pollutants” and highlight possible changes with time due to external factors (food contamination, transports).
- Assessment of pollutants body burden at different time, and consider the hypothesis of long-life accumulation of some persistent organic pollutants.
- Confirm the associations between exposure to some pollutants and some cardiovascular risk factors that was observed in ORISCAV-LUX 1.
- Investigate to what extent, the smoking ban in public places adopted in January 2014 in GDL, will impact the level of exposure to secondhand smoke in the general population.

3. RATIONALE

The ORISCAV-LUX 2 study constitutes an **inductive approach of research, a "bottom-up" method of reasoning, focuses on collected data to generate theories and hypotheses**. This project has dual significance: 1) it meets emerging needs for a sustainable health information system through collecting and establishing national baseline statistics for important cardiovascular pathologies and cardio-metabolic risk factors, 2) It constitutes a rich database attracting researchers, who are interested in cardio- metabolic and cognitive topics.

Knowledge and understanding of the epidemiological profile, in terms of body composition, environment and lifestyle-related traditional and emergent cardiovascular risk factors and associated pathologies, such as diabetes, hypertension, lipid disorders and metabolically healthy/unhealthy obesity/overweight/normal weight, is an essential pre-requisite to assess and address public health needs.

The Follow-up ORISCAV-LUX 2 study will allow to monitor the evolution of these potentially modifiable cardiovascular risk factors overtime. The findings would help to develop and plan for targeted prevention strategies. This small cohort will have the potential to provide stronger scientific evidences than the simple cross-sectional data. Risk factors are often linked with other causes and have their origin in a complex environment, consisting of socioeconomic factors, environmental and community conditions and individual behaviours. Drawing a causal chain shows the multiple entry points for interventions. Interventions may vary from introducing a new legislation, tax, financial incentives, health-promotion campaigns or engineering solutions. Reducing or eliminating risk factors through specific interventions could have a tremendous effect on disease burden and quality of life.

Understanding the pathophysiology and improving the clinical management of CVD also involve a knowledge of novel risk factors and biomarkers. Further research is needed to validate novel promising risk factors that will aid in the clinical evaluation and perhaps prediction of CVD in the adult population. This knowledge should eventually lead to the development of more directed therapeutic strategies to prevent CVD.

Finally, such creative, multidisciplinary research, represented by the ORISCAV-LUX 2 project, is extremely needed in the Department of Public Health, to address the research issues posed above and to design the major line of future research strategy, in line with "Performance

contract". This exciting research area would benefit from collaborative work of several in-house and international researchers.

4. INNOVATIVE RESEARCH ASPECTS

Specifically, the ORISCAV-LUX 2 project will contribution to the development of new knowledge regarding:

1. The association between *emerging* risk markers, for example, arterial stiffness and cognitive function, notably in older age group.
2. The association of *emerging* risk markers, for example, arterial stiffness with renal function in the general population.
3. The impact of vitamin D deficiency on cardiovascular health is well-documented, however, its influence on cognitive performance of the general population is totally unknown.
4. The association between structural vascular changes, measured by vascular adhesion molecules and angiogenesis) and traditional cardiovascular risk factors, particularly, smoking, alcohol consumption, dietary patterns, diabetes, hypertension, and obesity.
5. The association between structural vascular changes, measured by vascular adhesion molecules and angiogenesis) and emerging biomarkers as arterial stiffness, inflammation and thrombosis.
6. The association between structural vascular changes, measured by vascular adhesion molecules and angiogenesis) and cognitive impairment.
7. The association of stress biomarkers, measured by cardiac enzymes, and metabolic syndrome among the general population.
8. The longitudinal association of certain dietary patterns (measured via cluster analyses, dimension reduction technic, mixture model) with cognitive decline.
9. Environmental or occupational exposure to chemicals, such as solvents and lead, exerts direct neurotoxic effects on the brain and is associated with diminished cognitive functioning. So far, it is unknown whether exposure to organic pollutants, measured in the hair or blood samples, have a detrimental effect on cognitive performance.
10. Most of the data obtained from the general population are based on subjective measures (questionnaires). Using questionnaires do not give detailed and reliable information on the intensity, type and frequency of physical activity during the day. The objective measurement of physical activity will provide more data for in-depth analysis

of the physical activity level of the population, including the study of sedentary behaviour and sleep pattern.

11. The fitness level has been associated with the development of frailty and with an increased cardiovascular risk. As the level of physical activity is quite fluctuating, an objective assessment of fitness level will also help to better study the long-term physical activity patterns.

12. The physical function has been used as a proxy of frailty. Recent literature has highlighted the benefits of an objective measure to use as a frailty outcome.

13. Finally, with the integration of objective measures (physical functioning, cognitive, physical activity and fitness level) in ORISCAV-LUX 2, we could answer more accurately research questions concerning the development of frailty in the Luxembourg population, including physical and cognitive decline associated with determinants measured at baseline.

14. Metabolically Healthy / Unhealthy Weight Status: Recent studies defined metabolically healthy obese (MHO) people as individuals showing a healthy cardio-metabolic profile despite of having a high fat mass. Five other phenotypes were established according to the weight status and associated comorbidities: the metabolically unhealthy obese (MUO), the metabolically healthy and unhealthy normal weight (MHNW, MUNW) and overweight (MHOV and MUOV).

    Only few data concerning the prevalence of the metabolically healthy/unhealthy weight status are available in the previous published studies (MHO data, in particular). Moreover, studies about metabolically healthy/unhealthy phenotypes and associated comorbidities evolution over time remain controversial (for example for type 2 diabetes, mortality, etc.) and concern exclusively obese subjects.
    Furthermore, scarce studies tried to define the underlying mechanisms of the healthy / unhealthy metabolic weight phenotypes. However, these studies highlighted a reduced number of determinants (sugar-sweetened beverages, subjective measurements of physical activity [questionnaires]). Some others showed contradictory opinions, in particular in terms of body composition (lean/muscle mass involvement, for example).
    Finally, previous investigations still focused on MHO/MUO subjects, neglecting the MHNW, MUNW, MHOV and MUOV people. Nevertheless, independently of the weight, the healthy/unhealthy metabolic status should be identified to better manage cardio-metabolic health and improve statistical and clinical data interpretation in research. The

study of the Metabolically Healthy / Unhealthy Weight Status will bring some innovative knowledge on:

- The MHNW, MUNW, MHOV, MUOV, MHO and MUO prevalence and incidence (ORISCAV-LUX-1-2 samples)
- The metabolically healthy/unhealthy phenotypes determinants [objective measures of body composition, diet, physical activity, pollutants…] (ORISCAV-LUX-1-2 samples, in collaboration with the team)
- The metabolically healthy/unhealthy weight status evolution over time and associated risk factors and comorbidities (ORISCAV-LUX-1-2 samples)
- The relationship between the metabolically healthy/unhealthy weight status, body composition and potential frailty, respectively cognitive decline in the ORISCAV-LUX-2 sample (additional investigations in relation to Alaa and Gloria's thematics).

15. The relevance of hair analysis for the assessment of human exposure to organic pollutants was highlighted through several recent results from the Laboratory of Analytical Human Biomonitoring of LIH. Although some previous studies already conducted population exposure assessments, results demonstrated that each population have its own specificities regarding the pollutants people are exposed to, and hence the associated risks. As no such study has been carried out in Luxembourg yet, ORISCAV-LUX 1s the very first opportunity to provide exposure data on the Luxembourg population. As the previous studies in the field were based on the analysis of urine or blood, which were shown to be less reliable than hair for the biomonitoring of most compounds, the specificity of using hair for exposure assessment is a significant novelty of this project. Although a few studies were already conducted with hair analysis, most of them actually consisted in feasibility studies with limited number of chemicals tested in a limited number of individuals, or concerned specific populations mainly submitted to occupational exposure. The present study will therefore be the first one to involve such a large number of people representative of general population. The wideness of the list of pollutants simultaneously analysed here, including "novel" contaminants such as bisphenol and phthalates, will moreover provide a level of cumulative exposure assessment that had never been reached so far. The ORISCAV-LUX study is also the first one to investigate associations between biological adverse effects and exposure assessed by means of hair analysis.

The analysis of biomarkers of tobacco smoke in hair can be considered the most powerful tool for assessing both active smoking and secondhand smoke exposure in non-smokers on the mid-term. It provides objective value of the consumption magnitude in smokers, avoiding bias associated with self-declaration, and enables to document passive smoking in people that would categorize themselves as non-smokers. The first results obtained by LAHB for the Luxembourg population reported a significant level of second hand smoke exposure before smoking ban. Although some studies provided assessments of the effects of smoking ban on exposure and tobacco consumption, most were limited to specific contexts (catering staff, pregnant women…) and based on indirect evaluation. The follow up of the ORISCAV-LUX population will therefore provide the first assessment of the effect of smoking ban on exposure to secondhand smoke using objective and reliable means such as hair analysis, in a cohort representative of the country's general population.

16. Recent studies from the Laboratory of Cardiovascular Research at L.I.H. revealed the influence of cardiovascular risk factors on circulating levels of existing and emerging biomarkers. This finding originated from the analysis of a national cohort of patients with acute myocardial infarction. However, in biomarker studies using case-control design, there is also a need to address the issue of the influence of cardiovascular risk factors on circulating levels of biomarkers, and thus on their diagnostic value, in the control group. With the provision of such a control cohort, the present project will allow addressing this unresolved issue.A second major benefit of the project is to provide a biobank and a clinical database of controls that will be very useful for other translational studies performed in CVD patients. Of note, the ORISCAV-I population has already been used as control group in past studies performed at the Laboratory of Cardiovascular Research (153). With an ageing population, ORISCAV-II will provide a population with an average age closer to the average age of CVD patients.

    A third novelty is the use of the PAXgene system to collect blood samples. This system allows harvesting whole blood cells and extract high quality RNA without the need for immediate processing. This system is well suited for biomarker studies investigating RNAs and has not been widely used.

    Overall, another innovative aspect of the present project is to promote international collaborations with well-known research institutions (and thus to contribute to

enhancing L.I.H. international visibility), as well as internal collaborations within L.I.H. (7 labs involved).

17. The exposure to genetic risk factors, among cases with a particular disease and healthy controls is relatively new and has never been studied in Luxembourg's population. After appropriate ethical approval and signed informed consent the population-based ORISCAV-LUX 2 and potentially EHES participants will be the source of about 2000 healthy controls. Prevalent cases for a particular disease will come from hospital population. The need for very large sample sizes could be overcome by participating with Luxembourg's data into larger international studies, or by selecting cases with early age of disease onset.

    The use of complete DNA sequencing to scan for genetic variation gives the opportunity to discover Single Nucleotide Polymorphisms directly associated to a particular disease. Should be noted that given the prohibitive cost in the past, complete DNA sequencing has not been widely used.

    Given the available information from DNA sequencing, Mendelian randomization could be used to study the association between an environment exposure and a particular disease all by avoiding reverse causality bias.

5. STUDY OBJECTIVES

The ultimate aim of the Follow-up ORISCAV-LUX 2 study is to provide a valuable longitudinal national dataset to monitor cardio-metabolic health and answer specific research questions.

5.1. Primary objective (short term)

The primary objectives of the ORISCAV-LUX 2 study are:

- The assessment, at national level, of major traditional and emerging cardiovascular risk factors and associated conditions, such as diabetes, hypertension, lipid disorders,
- The description of temporal evolution of classical potentially modifiable cardiovascular risk factors between 2008 and 2016.

5.2. Secondary objectives (medium to long term)

These objectives correspond to post-project phase, which will be divided into several sub-projects, according to specific research questions, as suggested earlier by the in-house researchers.

For example:

Research Projects, suggested by Ala'a Alkerwi

Potential Title	**The incidence of CVD-associated pathologies in Luxembourg**
Objectives	This sub-project has several major objectives: 1. Investigate the new cases (incidence rate) of CVD-associated pathologies, such as diabetes, hypertension, dyslipidaemia, obesity, metabolic syndrome, 2. Assess the prevalence of *emerging* cardiovascular risk pathologies, such as arterial stiffness and inflammatory and thrombotic profile, 3. Assess the prevalence of early cognitive impairment among the general adult population in Luxembourg.

Potential Title	**The impact of CVD risk factors on cognitive function**
Objective	Investigate the cross-sectional relationship between *traditional* (hypertension, diabetes, smoking, alcohol behaviours) and *emerging* CVD risk factors (arterial stiffness/PWV) and cognitive impairment. The full-proposal of this project will be written in detail for a grant from FNR/CORE program.
Potential Title	**Exploring food-cognition link**
Objectives	Investigate whether specific dietary behaviours/ specific dietary patterns may affect cognitive function, with a particular focus on diet quality and specific nutrients. The full-proposal of this project will be written in detail for a grant from FNR/CORE program.
Potential Title	**Exploring environmental exposure (pollutants) and cognitive function**
Objectives	Investigate whether specific environmental chemicals may affect cognitive function. The full-proposal of this project will be written in detail for a grant from FNR/CORE program.

Research project (s), suggested by Hanène Samouda

Potential Title	**The metabolically healthy/unhealthy weight status: Prevalence, incidence, determinants and associated comorbidities**
Objectives	1. Investigate the prevalence / incidence of the metabolically healthy/unhealthy normal weight, overweight and obesity (2007-2015). 2. Define the determinants [body composition, food habits, physical activity, sedentary lifestyle, pollutants] of the 6 metabolically healthy/unhealthy weight status phenotypes and their evolution over time (2007-2015). 3. Analyse the potential transition/evolution between the metabolically healthy/unhealthy weight status phenotypes (2007-2015) and the associated causes. 4. Analyse the body composition [fat mass, muscle mass, visceral adipose tissue] of the metabolically healthy/unhealthy normal weight, overweight and obese phenotypes. 5. Investigate the relationship between the metabolically healthy/unhealthy weight status and cognitive decline 6. Investigate the relationship between the metabolically healthy/unhealthy weight status and the healthy ageing / frailty 7. Investigate the relationship between body composition [fat mass, muscle mass, visceral adipose tissue] and cognitive decline,

Research project, suggested by Gloria Aguayo

Potential Title	**The role of physical activity as determinant of frailty in elderly Luxembourgish general population**
Objectives	In cross-sectional analysis 1. Assess physical activity and sedentary behaviour with objective measurements and compare with subjective measurements (IPAQ questionnaire) 2. Evaluate the fitness degree as variable associated with physical functioning and other outcomes such as diabetes, obesity and cardiovascular disease. 3. With validated scores, investigate the prevalence of frailty in Luxembourgish population. In longitudinal analysis: 1. Define the role of physical activity at baseline as determinant of frailty, assessed with physical functioning measurements. 2. Determine the physical activity mode (intensity, duration, type) that better explains the association with physical functioning after 7 years of follow-up. 3. -Determine the role of sedentary behavior at baseline as determinant of poor physical functioning.

Research project, suggested by Brice Appenzeller

Potential Title	**Exposure of Luxembourg population to organic pollutants**
Objectives	1. Assess the exposure of Luxembourg population to organic pollutants by hair, urine and blood analysis 2. Compare information obtained from the different biological matrices 3. Study the evolution of individual exposure by repeated measures 4. Study associations between cardiometabolic risk factors and exposure to pollutants
Potential Title	**Influence of smoking bans on tobacco consumption habits and passive smoking in Luxembourg**
Objectives	Assess the prevalence of active and passive smoking by hair analysis in Luxembourg before and after smoking ban in public settings in order to highlight the influence of law on tobacco consumption and passive exposure.

Research project, suggested by Yvan Devaux

Potential Title	Association between CV risk factors and cardiac biomarker levels
Background	The association between CV risk factors and the levels of cardiac biomarkers has been poorly investigated, yet it represents a serious limitation to biomarker studies.
Objectives	1. Investigate the association between CV risk factors and the circulating levels of traditional and emerging (e.g. microRNAs, long noncoding RNAs) cardiac biomarkers. 2. Investigate the influence of the association between CV risk factors and the circulating levels of cardiac biomarkers on their predictive value. 3. Provide a biobank and a clinical database for use as control of other translational studies performed in CVD patients in Luxembourg.

Research project, suggested by Dritan Bejko

Potential Title	**Genetic predisposition for several complex chronic diseases**
Objectives	1. Investigate the association between genetic predisposition and Coronary Heart Disease susceptibility 2. Investigate the association between genetic predisposition and susceptibility to other complex chronic diseases.
	Genome Wide Association Studies (GWAS) will be used to study the exposure to genetic risk factors among cases with a particular disease and healthy controls. To study genetic predisposition to Coronary Heart Disease (CHD) the incident/prevalent cases will be selected among patients diagnosed with a CHD in Luxembourg's Hospitals. Age, sex and ethnicity matched CHD free (healthy) population based- controls will be selected from the ORISCAV-LUX 2 study participants. The same design could be used to study obesity, diabetes, Hypertension, etc. To scan for genetic variations (Single Nucleotide Polymorphisms) the gene chips (Micro array) will be used. Complete sequencing would also be a viable option given the continuous drop in the costs of that technique. By controlling for population stratification (using the self-declared ethnicity or genetic markers), analysis of generated data could help identify SNP-s that are more frequently found among cases than among disease free controls. Given the need for large samples inclusion of healthy controls from participants of other studies or from healthy blood donors is an option that has been successfully used elsewhere. Participating with Luxembourg's data into larger European studies would be also another possibility. With the advances of technologies the benefits for the patients could range from personalised preventive medicine, to treatments tailored to patient's genetic make-up.

6. MATERIAL AND METHODS

6.1. Study Design

The ORISCAV-LUX 2 survey is designed as a 7-year follow-up study. Therefore, the first wave participants will be re-contacted to take part in the second wave.

The following figures show which kind of data collection will be made in the follow-up study in comparison to the inclusion period. (cf. Figure 2 and Figure 3)

	Items	Wave 1	Wave 2
SELF-ADMINISTERED QUESTIONNAIRE	Socio-demographic variables	+	+
	Questions on occupation	+	+
	Quality of life	-	+
	Mental well-being	-	+
	Social support	-	+
	Nutritional habits	-	+
	Physical activity	+	+
	Sleep quality	-	+
	Vitamin D intake	-	+
	Thyroid health	-	+
	Health of digestive process	-	+
	Pollution	-	+
	Tobacco consumption	+	+
	Nicotine dependence	-	+
	Alcohol	-	+

Figure 2 The content of the self-administered questionnaire and the differences between both study waves

	Items	Wave 1	Wave 2
INTERVIEW WITH RESEARCH NURSE	CANTAB	-	+
	MMSE	-	+
	Autonomy (ADL and IADL)	-	+
	Personal medical history	+	+
	Medication and supplements	+	+
	Vitamins intake	-	+
	Food Frequency Questionnaire	+	+
	Weight, height, waist and hip circumference	+	+
	Proximal thigh circumference	-	+
	Impedancemetry	-	+
	Blood pressure and pulse	+	+
	Cardiovascular history	-	+
	Electrocardiogram	-	+
	Pulse wave velocity	-	+
	Hair sample (≥200mg)	+	+
	Guthrie card	-	+
	Blood and urine samples	+	+
	Finger tapping test	-	+
	Grip strength test	-	+
	Balance test	-	+
	Chair rising test	-	+
	Walking speed	-	+
	Step test	-	+
	Accelerometer	-	+

Figure 3 The content of the nurse interview and the differences between both study waves

6.2. Study population

Similar to the first wave, the target population represents the individuals residing in Luxembourg. After 7 years interval, now they are aged between 25 and 76 years. The entire sample is eligible to participate in the second wave ORISCAV-LUX study. Subjects deceased before second recruitment will be registered in the study database.

6.2.1. Subject inclusion criteria

The follow-up project will be based on ORISCAV-LUX wave 1, which is composed of 1432 subjects. Inclusion criteria are: 1) Being participant to ORISCAV-LUX 1, and 2) Informed consent obtained and signed.

6.2.2. Subject exclusion criteria

All participants will be re-contacted and recruited for the second wave, except:

- Pregnant women
- Those who have announced their refuse after taking part to wave 1 (n=16).
- Those who are institutionalised like in hospital, jail, nursing home, elderly home
- Those who are not anymore a resident of the Grand-Duchy of Luxembourg
- Those who are not able to visit the examination site for any medical reason

6.3. Timing of the survey

Taking into account the fact that some people may not wish to take part or drop out and that the study team may take vacations or be absent, the enrolment of 900 to 1000 participants for the whole study period seems realistic. It is planned to carry out the enrolment phase in 15 months between July 2015 and September 2016.

6.4. Preparation of the study procedures

Various elements related to the operational conduct of the study will initially be prepared:

6.4.1. Ethical considerations

Ethical approval of the study protocol, by the CNER (National Committee of Research Ethics), shall be obtained in May 2015, followed by the CNPD (National Commission for Private Data Protection) notification in May 2015.

Participants will have the choice to decide whether they want to take part only in the present follow-up study or also in future studies including genotyping analysis. Therefore the informed consent has been adapted accordingly. For this purpose, new study protocols will be submitted in a later step for CNER approbation. An authorisation from the CNPD shall be received as well before any genotyping analysis.

6.4.2. Study related communication and media coverage

Along with the study kick-off, a media coverage campaign, targeting the general public and health professionals, will be launched at national level. Posters and leaflets will be widely distributed to encourage the previous participants to take part in the study. At the same time, the website www.ORISCAV-LUX.lu will be updated, prior to the start of the field phase.

6.4.3. Instrument translation

All documents, including questionnaires, invitation letter, reply-coupons, information leaflet and consent form, will be translated into 4 languages (French, German, Portuguese and English) to ensure participants' comprehension and promote their participation.

6.4.4. Staff training

The study personnel (nurses and administrative agent), will be trained prior to the start of the field stage, to perform accurately the assigned tasks. For each test and medical exam a standard operating procedure will be written. Each nurse is asked to apply the procedures.

The training covers the following major aspects:

- An introduction to the general context of the study topic, the current state of knowledge of cardiovascular risk factors, and the principal objectives of the study will be presented. For this specific aspect, all team members, including data manager, statistician, IT specialist, will participate;
- Information about the recruitment workflow as wells as lab flow will be communicated
- Information about the ethical aspects, including the importance of confidentiality, participant's security, and private data protection will be given;
- Nurses will acquire the questionnaires content knowledge. Procedures for data collection quality will be reminded.
- For each test and medical exam, nurses will benefit of a specific and adapted training;

- The administrative agent will acquire the skills to perform the phone calls, establish a relationship of trust and gain the cooperation of the respondents according to a pre-defined procedure;
- Three procedures for quality control will be explained to the team (anthropometric, participant's file, equipment)

6.4.5. Logistical preparation

All necessary equipment will be purchased and prepared prior to the launch of the field stage.

6.4.6. Preparing the recruitment centres

In order to ensure a smooth running of the study, three recruitment centres dispatched all over the national territory (Strassen, Esch-sur-Alzette, Ettelbruck) will be proposed to the participants.

6.4.7. Creating the data entry template

There will be two different questionnaires:

- First, participants are asked to fill a questionnaire at home. They can choose whether they want a paper form or preferably an online form.
- Secondly, there will be a questionnaire to be filled in by the nurse.

The online questionnaire will be developed on LimeSurvey software and the nurse questionnaire on Clinsight ® software.

6.4.8. Selection of the analytic laboratory

An agreement with a private laboratory that owns antennas throughout the whole country is currently under negotiation. The private laboratory would be responsible in collecting the biological samples, performing the analysis. The locations and services of this private laboratory are well-known and accepted by the overall population.

This laboratory has been accredited according to ISO standard 15189, which is a specific standard for clinical testing laboratories, for the tests laid down in the accreditation scope in the spheres of autoimmunity, biochemistry, haematology, hormonology and microbiology.

6.5. Recruitment phase

6.5.1. Stages of participation

Consistent with the WHO's Step-wise strategy, the participation to the ORISCAV-LUX 2 study includes 3 main steps (Figure 4):

Step 1 : filling in a self-reported questionnaire

Step 2 : measurement of anthropometric parameters (weight, height, waist, hip and proximal thigh circumferences), blood pressure, pulse rate, ECG, pulse wave velocity (Complior ®), body composition (Tanita segmental body composition analyser BC 418), physical function (GENEActiv® accelerometer), as well as mental function (CANTAB®), according to standardised operating procedures.

Step 3: blood and urine sample collection at laboratory

Figure 4 The WHO's STEPS-Wise strategy for monitoring risk factors (2001)

6.5.2. Procedure of subjects recruitment

The participant's recruitment procedure will comprise several stages as depicted in Figure 5.

6.5.2.1. Sending an invitation letter

The participants will receive an invitation letter together with the information leaflet, a coupon-answer and a pre-paid envelop, proposing them to take part in the second wave of the

ORISCAV-LUX survey. Volunteers willing to participate can directly fill in the questionnaire online accessible with a unique identification code. Those who prefer a paper version can simply send back the coupon-answer indicating their preferred language. The administrative agent will then handle the mailing of the paper questionnaires to the participants.

The recruitment follow-up will be managed with an Access software® recruitment database.

If no response is received after 21 days, the non-respondents will receive a second invitation letter. A final attempt to contact the selected people will be made eventually by phone if no answer has been received after additional 21 days.

6.5.2.2. Phone contact and appointment

The consenting subjects will rapidly be contacted by phone, by the administrative agent, to schedule the nurse appointment (see table 1) at one of the nearest study centres. Appointments can be scheduled as follows:

- Subjects are welcome between 6 am and 10 pm from Monday to Friday.
- Every two weeks appointments on Saturdays are optional

After each appointment scheduling, on a daily basis, the administrative agent will manage the mailing of confirmation letters. In addition to the venue details, the participant will also get specific instructions related to medical exams.

6.5.2.3. Conduct of field work

The survey duration for one participant is foreseen to take about 4 hours.

Before the participant's arrival the nurse has to get prepared:

- Printing out of identification labels, if existing the electronic self-reported questionnaire
- Ensuring the reception of the paper self-reported questionnaire
- Preparation of the examination room

After the participant has arrived at the examination site, the study nurse will, first of all, explain the survey, ensure that the participant understands the provided information and has the adequate decision capacity. The study nurse will ask the participant to sign the informed

consent form in duplicate. Only once the participant has agreed to take part in the survey and signed the informed consent form, medical examinations can start.

Table 1 Appointment timetable of the research nurse with a participant

	Research nurse appointment	Time table	
MOD0	Preparing room and quality control	00:05:00	
MOD1	Information letter and consent form signature	00:05:00	
	Validation of self-reported questionnaire	00:10:00	
MOD2	Cognitive tests (CANTAB + MMSE), ADL and IADL questionnaires, Personal history, Medication and supplements intake, Vitamins intake	00:50:00	
MOD3	Food frequency questionnaire	00:45:00	MOD 1/2/3
			01:55:00
MOD4	Anthropometry, impedanciometry	00:08:00	
	Blood pressure	00:05:00	
	Cardiovascular history	00:05:00	
	ECG	00:08:00	
	Pulse wave velocity (arterial stiffness)	00:30:00	
MOD5	Samples: hair (≥200mg), blood (guthrie card), Lab appointment to plan (blood + urine)	00:10:00	
MOD6	Finger tapping	00:02:00	
	Grip strength	00:02:00	
	Balance	00:02:50	
	Chair rises	00:05:00	
	Walking speed	00:02:50	
	Step test with Actiheart	00:08:00	
	Accelerometer	00:10:00	MOD 4/5/6
			01:38:40
MOD7	Farewell participant with results coupon	00:04:00	
	Case closure check list	00:01:00	TOTAL
TOTAL	**Duration of appointment**	**03:33:40**	03:33:40
MOD0	Cleaning room, data entry and	00:10:00	
	Preparing upcoming appointment		

6.5.3. Pilot phase

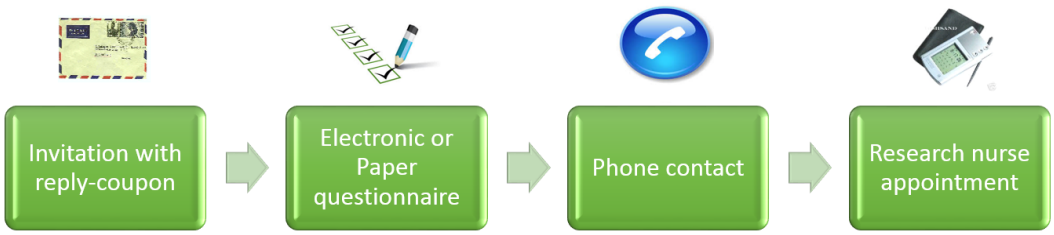
Before starting the recruitment of the ORISCAV-LUX 2 sample, a pilot phase between May and June 2015 to test the study procedures will be organised. It will help to readjust the Fieldwork procedure in case of potential difficulties. Then, the enrolment phase will then take place during the next 14 months to be in line with the first wave of the project.

Ten volunteers, aged from 25 to 76, will be asked to help us to assess the recruitment process. Each time two nurses will be together for one single appointment: a first nurse will conduct the examination while the second one will observe and evaluate the process.

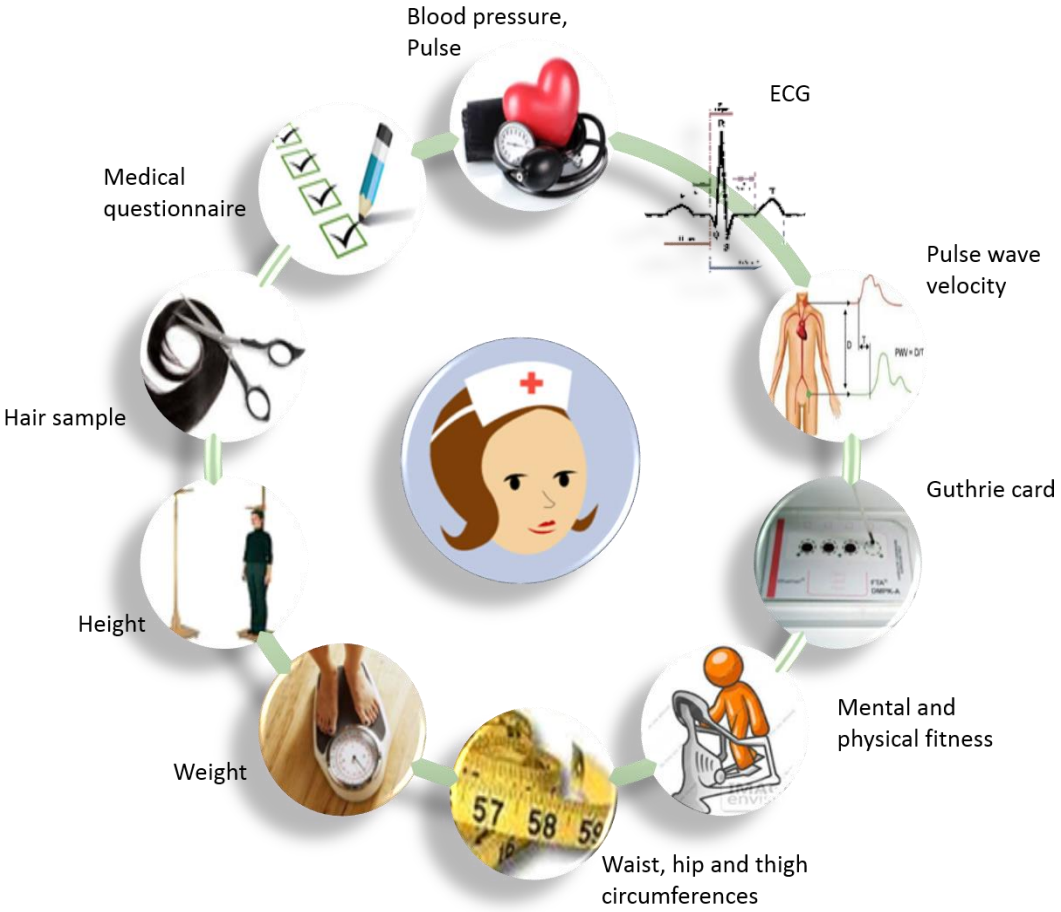
The pilot phase analysis is scheduled in June and potential adjustments and decisions will be made accordingly.

Figure 5 Schematic representation of the survey participation process

1. AT HOME



2. DURING THE RESEARCH NURSE APPOINTMENT



3. AT THE LABORATORY

6.6. Questionnaire design and contents

The ORISCAV-LUX 2 questionnaires are divided into 2 separated questionnaires: a self-reported questionnaire to be filled by the participant at home and another one is focused on the medical aspects which will be completed by a nurse. Together both questionnaires are covering the following items

6.6.1. Demographic and Socioeconomic information

- Demographics: age and sex
- Country of birth
- Marital status
- Education level (highest diploma)
- Professional situation, type of profession
- Objective monthly household income and number of adults, children
- Subjective evaluation of economic resources
- Health perception

6.6.2. Lifestyle questionnaires

- Tobacco consumption (Fägerstrom questionnaire)
- Alcohol intake (AUDIT questionnaire, by the WHO)
- IPAQ (International Physical Activity Questionnaire)
- Dietary habits (FFQ - Food Frequency Questionnaire)

6.6.3. Health status module

- Quality of life (SF-36 questionnaire)
- Evaluation of autonomy (ADL and IADL instruments)
- Mental well-being (CES-depression)
- Social support
- Sleep habits (PSQI questionnaire)

- Vitamin D questionnaire
- Thyroid function questionnaire
- Constipation (CSS questionnaire)
- Personal general health status
- Cardiovascular history
- Pollution
- MMSE-2 test
- Personal diseases and chronic conditions
- Medication and supplements intake
- Vitamins intake
- Family history

6.7. Clinical measurements

6.7.1. Blood pressure and pulse rate

Pulse rate, systolic and diastolic blood pressure will be measured at least three times at 5 minutes interval, according to the same Standard Operating Procedure used in wave 1.

6.7.2. Anthropometric measurements

According to the same Standard Operating Procedure used in wave 1, Height (cm), Weight (Kg), Waist, and Hip circumferences (cm) will be measured. Proximal thigh circumference (Proximal Thigh C) will be assessed by a distal positioning of the measuring tape to the gluteal crease and around the thigh. Body Mass Index (BMI) and Waist−to−hip ratio (WHR) will be calculated. Visceral adipose tissue (VAT) will be predicted according to the Samouda *et al* (2013) models (154).

6.7.3. Bio-impedance analysis

Total and segmental [right arm, left arm, trunk, right leg, and left leg] body composition [fat mass (FM), fat free mass (FFM) and predicted muscle mass (MM)] will be assessed by the Tanita segmental body composition analyser BC 418. The participant will be standing, on the weighing

platform with the heels placed on the posterior electrodes, the front part of the feet in contact with the anterior electrodes and grasping the grips with both hands(155), (156).

6.7.4. Cardiac function

6.7.4.1. Electrocardiogram (ECG)

Cardiac function will be assessed by performing an ECG on every consenting participant. The ECG recordings will serve to detect cardiac abnormalities and cardiomyopathies such as cardiac hypertrophy, auricular fibrillation, prolonged QT interval, and arrhythmias.

6.7.4.2. Pulse wave velocity (PWV)

Arterial stiffness, as measured by carotid-femoral PWV, will be assessed, by using Complior® apparatus. Carotid-femoral pulse wave velocity (PWV) has been proposed as the gold standard for arterial stiffness measurement.

6.7.5. Cognitive function

Cognitive function will be asses by using the CANTAB® Core Cognitive Battery, a fast, accurate and easy to administer cognitive assessment system. It is a kind of computerized cognitive testing, designed specifically for use in academic research studies. The battery is composed of three test:

- Reaction / Time CANTAB Reaction Time (RTI);
- Episodic Memory/ CANTAB Paired Associates Learning (PAL);
- Working Memory CANTAB Spatial Working Memory (SWM).

These tests, along with the MMSE, are ideal for assessing all areas of general cognition and acutely sensitive to even the most subtle changes in cognitive function. These language-independent tests and touch screen technology deliver rapid and non-invasive cognitive assessment, designed to minimize sources of bias that may affect performance.

6.7.6. Physical function

Physical functioning will be based on two types of measurements:

1. Subjective measures (questionnaires) : SF-36 (physical domain of quality of life) and ADL and IADL (disability)

2. Objective measures (physical examination) which is in line with the protocols of the English Longitudinal Study of Ageing (ELSA). These measures include:

   - Walking speed (measured by a trained nurse, following a standardized protocol). Measured in m/s

   - Chair rises (time to complete 5 chair rises: participants sit on an armless chair with feet resting on the floor and arms folded across their chest. They stood up without using their arms and sat down five times as quickly as possible). Measured in s/5 times.

   - Balance (assessed through a series of tests of varying difficulty: full- and semi-tandem stands, one-leg balance with eyes open or closed). Measured in seconds.

   - Finger tapping (the number of taps during 10 s was recorded using an electronic device (WPS Electronic tapping test)). Measured in taps/10s

   - Grip strength (in kilograms; dominant hand, measured using a Smedley handgrip dynamometer). Measured in kg,

6.7.6.1. Cardiorespiratory fitness

Cardiorespiratory fitness will be measured in all participants without contraindications (medical contraindication, orthopaedic problems or not able to climb stairs) and not taking heart rate regulating medications (e.g. beta blockers) using a validated step test (157). The prediction of VO2 max will be estimated with the step test using a 20 cm platform and Actiheart (CamNtech Ltd. Cambridge, UK), a compact device that simultaneously records activity and heart rate and uses both parameters to calculate activity energy expenditure. Actiheart software provides individual heart rate calibration data for the step test and has been validated against Doubly Labelled Water (158).

Physical activity will be measured with accelerometers. The participants will use a physical activity tracker during 7 days using (GeneActiv; Activinsights Ltd., Cambs, UK) a wrist-worn, tri-axial, raw data accelerometer, allowing the measuring of physical activity and sleep patterns with a temperature sensor and a frequency of 100Hz or higher. This device shows good compliance in the population and has been used in general population study cohorts(159), (160), (161).

6.8. Biological measurements

During the second visit at the laboratory, the following biological samples will be taken from participants:

6.8.1. Blood

The following biomarkers will be measured in the fasting blood samples, in addition to core biological measurements done in the first wave of the ORISCAV-LUX study:

- Glycaemic biomarkers: Glucose, Glycated haemoglobin (HbA1c), Insulin
- Lipid biomarkers: Triglycerides, Total and HDL cholesterol (LDL cholesterol calculated, Apolipoprotein A and B)
- Inflammatory biomarkers: hs-CRP
- Liver enzymes gamma GT, GOT, GPT
- Renal biomarkers: Creatinine, uric acid
- Thyroid function tests: TSH, T3, T4
- Blood formula (Complete Blood Count CBC)
- Albumin
- Ferritin
- Electrolytes (Na, K, Mg, Ca)
- Vitamin D
- Cardiac biomarkers

In addition, 5 ml of serum sample will be frozen for several toxicological biomarkers including persistent organic pollutants (POPs) dosage, which will be done at a later date.

The following POPs will be dosed:

- 14 polychlorinated biphenyls (PCBs): dioxin-like PCBs—CB105, CB118, CB156, CB157, CB189; nondioxin-like PCBs—CB74, CB99, CB138, CB153, CB170, CB180, CB194, CB206, and CB209;
- 5 organochlorine pesticides (Ops): HCB (hexachlorobenzene), trans-chlordane, cis-chlordane, trans-nonachlor and 2,2-Bis (4-chlorophenyl)-1,1-dichloroethene);
- 1 dioxin: octachlorodibenzo-p-dioxin;
- one brominated diphenyl ether (2,2',4,4'-tetra-bromodiphenyl ether).

In addition, 12.5 mL of blood will be collected for novel biomarker studies: 2.5 mL will be drawn in PAXgene RNA tubes for extraction of total RNA from whole blood cells, 5 mL will be drawn in citrated tubes for plasma isolation and 5 ml will be drawn in dry tubes for serum isolation. PAXgene tubes will be stored at -20°C until RNA extraction. Plasma and serum samples will be aliquoted and stored at -80°C.

During the fieldwork appointments, the nurses will collect Dried Blood Spots of participants (DBS collection kits from LipiDx also known as Guthrie card) for genotyping and Polymerase Chain Reaction (PCR). Specifically a specimen of 5 pre-printed circles filled with blood spots will be done. Air-dry blood spots will be stored in envelopes and archived in a secure place at L.I.H. for subsequent analysis.

6.8.2. Urine

Creatininuria and Microalbuminurea will be measured in the urine. For further analysis of pollutants, 10 ml of urine will be collected and frozen in a glass tube at -80°C.

6.8.3. Hair

The following biomarkers will be analyzed in the hair specimens:

- Polycyclic aromatic hydrocarbons (PAHs)
- Various pesticides
- Nicotine and cotinine (smoking)

- Ethyl glucuronide (alcohol consumption)

These analyses will allow the examination of occupational and environmental pollution and their relation to the morbidity profile.

During the fieldwork appointment, a quality control will be done with a specific scale (mg) to assure a minimal quantity of hair which corresponds to ≥200mg.

6.9. Feedback to the participants

At some stage during the informed consent procedure, the participant will be asked if she/he agrees to receive a feedback of her/his results. Additionally, the participant can authorise the L.I.H. to send a copy of the results to a medical doctor of her/his choice (general practitioner, family doctor or any referent medical doctor).

A convention with two cardiologists from the Centre Hospitalier de Luxembourg (CHL) will be signed in order to assess ECGs from all the participants in this second wave and verify the presence or not of anomalies. Participants with an ECG result will be asked by the nurse if they want or not that the cardiologists could have access to the name of their physicians. In urgent case, the cardiologist of the study will obtain the name of the participant's doctor for potential discussion. The cardiologist will give recommendations but is not allowed to prescribe a treatment or any complementary exams.

The cardiologists will also interpret and comment on routine laboratory results. The feedback procedure will depend on the medical evaluation done by the cardiologists who will classify the results into 3 different categories: normal, abnormal and seriously abnormal. The feedback letter will include the results and a synthesis signed by the project leader, the medical investigator and the field work coordinator.

1. Normal: feedback in a letter sent to the participant (if the participant agreed).
2. Abnormal: feedback in a letter sent to the participant in any case. The participant is invited to contact his medical doctor for prescriptions or complementary exams.
3. Seriously abnormal: feedback communicated by phone and in a recommended letter sent to the participant in any case. The participant is invited to contact immediately his/her medical doctor. In case the results are seriously abnormal, the participant's medical doctor will be informed orally.

A copy of the feedback letter will be automatically sent to the participant's medical doctor if the participant gave his consent to do so.

In sum, the results of the routine biological analysis, clinical (blood pressure and ECG) and anthropometric measurements (weight, height, waist circumference and BMI) will be communicated to the participants.

7. PATIENT'S COMPLIANTS AND SAFETY MEASURES

Safety guidelines related to each measurement will be developed and included in the survey manual. The fieldwork team is trained in first aid and in calling the official emergency aid (dial number 112).

Adverse events (AEs) emerging on the way to or from the examination site and at the examination site will be reported to the fieldwork coordinator which has to inform orally the principal investigator and formally in a written form the medical investigator as well as the Head of Department. The Quality Control Unit will get each event described on the corresponding Standard Operating Procedure (SOP) and the unit will evaluate the AEs and assess if the event should be notified as a serious adverse event (SAE)(162).

The broad definition of an SAE as defined in the International Conference on Harmonization (ICH) guidelines includes any event that:

- is fatal
- is life threatening, meaning the subject was, in the view of the medical investigator, at immediate risk of death from the reaction as it occurred
- is a persistent or significant disability or incapacity, i.e., the event causes a substantial disruption of a person's ability to conduct normal life functions
- requires, or prolongs inpatient hospitalization
- is a congenital anomaly or birth defect

All events considered as SAEs should be notified with a complete report within 24 hours to the medical investigator and within 14 days to the ethics committee(162).

8. DATA MANAGEMENT

The Competence Centre for Methodology and Statistics (CCMS) at L.I.H. will be involved in developing and setting up the data management system.

Data entery and data management will be performed using the Clinsight® software version 7. Clinsight® stores the data in an Oracle® databases located on an independent server. Study team members such as the data manager, the statistician, the study nurses and the project leader have been specifically trained and are already experienced in using this program.

LimeSurvey® will be used to collect the data of the self-administered online questionnaire.

8.1. Data management processes

Fieldwork data will be collected through self-administered questionnaires, interviews, clinical examinations and biological analyses. Therefore, there will be three different kinds of data collected (Figure 6):

- Self-administered web or paper questionnaires filled out by the participant
- Case Report Form (CRFs) (anthropometric and clinical measurements) filled out by the fieldwork team
- Electronic files (biological analysis measurements, including laboratory results, CANTAB, Complior; Tanita segmental body composition analyser, ECG, GeneActiv accelerometer).

Fieldwork data will be saved in a Clinsight® database. Consistency checks will be run on the database and their results saved in the corresponding module of Clinsight®.

Survey sample of ORISCAV-LUX 1, including nominative data transferred from the IGSS, is currently stored separately in an Excel® database under the responsibility of the Principal Investigator. This nominative data base will be transformed into MS Access ® 2013 database, called as “Recruitment database”. These data issued from sample selection and recruitment includes sampling data, contact and re-contact of invited people, data on appointment schedules, non-participant reasons will be stored in a secured location. Only authorised staff members will have access to the recruitment database. The fieldwork and quality control variables will be stored in a Clinsight® database.

Data from paper CRFs will be double entered into the database. Data from the self-administered web questionnaire as well as laboratory data will be imported into the same database. In a later stage, checks will be programmed to test the entire fieldwork database for completeness, outstanding values and overall consistency in a later stage (Figure 6).

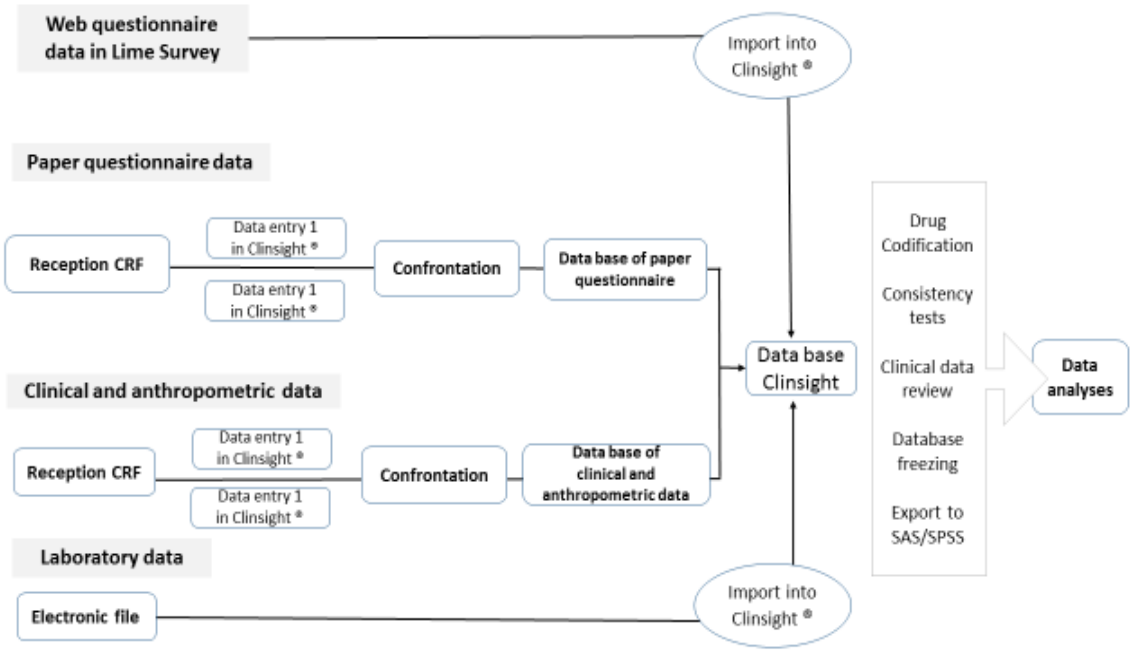


Figure 6 Data management processes

8.2. Data Handling

The source documents will be anonymized. Three different anonymised numbers will be used:

- An internal number
- A number for biological data from Ketherthill
- A research number which is generate only if the person accepts to participate

The internal number will be used to identify participants in the recruitment database. The research number will identify the survey participant on each source document. The research number will be linked to the participant via a separate identification list, called the “Recruitment database” which contains nominative information of each participant, the internal number, the

Ketherthill number and the id used in the first wave of ORISCAV-LUX. This list will be maintained in a secure place and accessible to specific team members only under the responsibility and management of the principal investigator(162).

8.3. Data security

Data security (confidentiality, integrity and availability) will be ensured throughout the entire data management process.

8.4. Data confidentiality

Clinsight® and LimeSurvey applications feature an access control list (ACL) and role system that allows defining individual data access rights to each survey member according to its responsibilities. The system prevents the disclosure of information to unauthorized individuals or systems. External access to the system is provided either:

- With a full Clinsight® client ran through Citrix® technology, using a strong authentication system (password, pin code and physical token). Access to Citrix® is made through a secured 128-bits encrypted HTTPS connexion.

- With a web interface. Access to the web interface is made through a secured 128-bits encrypted HTTPS connexion. The dedicated web server is hosted in a secured DMZ(162).

8.5. Data integrity

All survey data is physically stored in an Oracle® 11g database on a dedicated server located in the L.I.H. No direct access to this database is allowed. Any data entry/modification is made through Clinsight® and is automatically logged to a non-changeable audit trail. This helps to track and safeguard the accuracy and completeness of information and processing methods from intentional, unauthorised, or accidental changes. LimeSurvey® data is stored in a separate mySql database server.

The server that hosts the database features a redundant storage system (RAID hard drives) that prevents any physical breakdown.

A daily full backup of the database is made. Backup files are stored on hard drives and tapes. Backup tapes are kept in a secured distant place. The server room is protected against fire and heat (162).

8.6. Data availability

All involved servers (database, application, etc.) are virtualised. In case of any physical breakdown of a host server, virtualised servers can easily be transferred in real-time to another host. All active network components are redundant. This infrastructure ensures the high-availability of the system.

8.7. Record retention

The medical investigator and project leader have the responsibility to keep all databases on file for at least 10 years after completion or discontinuation of the study.

The fieldwork coordinator collects all signed consent forms and all questionnaires from the beginning of the recruitment phase until the end, and manages the archive of these files in a secure place at L.I.H.

9. STATISTICAL CONSIDERATIONS

9.1. Sampling design

For the first wave of the ORISCAV-LUX study, the adequate sample size was calculated to insure a national representativeness of the adult population residing in Luxembourg, so that to correctly assess the prevalence of various potentially modifiable cardiovascular risk factors among the non-institutionalized general adult population, aged 18-69 years.

Briefly, a representative random sample was drawn from the national health insurance registry, stratified by gender (male and female), age (5-year categories) and geographic districts of residence (Luxembourg, Diekirch and Grevenmacher). With a 98% social coverage rate, the registry is considered as the most complete list of inhabitants available in Luxembourg. The minimal sample size was calculated to 1285 subjects to ensure statistical power(163). However, based on literature review and previous evidence with such multiple-stage population-based studies, a high non-participation rate was expected, including refusal, invalid addresses and non-response. Assuming a response rate of 30% and a proportion of 5% of institutionalized subjects in each stratum, the sample size was augmented to 4496 subjects. The distribution of selected subjects in each stratum was proportional to their distribution in the source population (adult population of 18 to 69 years residents in Luxembourg). Pregnant women, people living in institutions, subjects outside the age range 18-69 years and those deceased before recruitment were excluded(125), (126).

Between the 9th of November 2007 and the 15th of January 2009, we succeeded to enroll 1432 participants, i.e., 32.2% participation rate which represents a realistic rate for this type of survey, and corresponds to the expected rate, upon which the sample size was calculated.

9.2. Expected sample size

For the second wave, it is expected to recruit about 900-1000 participants, taking account of the non-participation. Several reasons may contribute to loss of participants to follow-up; formal refusal (16 participants), unavailability due to lack of time, movement or immigration, problems of false addresses, death. Therefore, it is planned to perform all the possible strategies to increase the participation, for example, repeated contacts, explanation and motivation, feedback about clinical results.

9.3. Sample size calculation

In this second wave, failure to recruit the entire sample may limit our ability to correctly assess the new incidence cases and also to make generalizations to the general population (i.e., limit our ability to make statistical inferences).

Therefore, the adequate sample size to answer several main research questions has been calculated with the help of our in-house CCMS service to insure the power of specific sub-studies.

Healthy / Unhealthy Metabolic Weight Status Phenotypes:

Part1: Incidence / Prevalence of the Metabolically Healthy/Unhealthy Phenotypes:

Based on published studies (43), (164) , we can expect the following phenotypes prevalence in Luxembourg: 25% MHNW, 20% MHOV, 10% MHO, 7% MUNW, 15% MUOV and 20% MUO. We assume that the ORISAV-LUX II Survey would allow these phenotypes subgroups prevalence's to be showed with a 95% confidence interval and a precision of at least 3%. The required minimal sample size would thus be 801 participants. With N=1432 subjects that took part in the ORISCAV-LUX survey, precision would be increased.

Part2: Identification of the Metabolically Healthy/Unhealthy Weight Status Determinants and their evolution over time:

Following determinants distribution previously published in the literature (43), if we assume that the odds ratio to be metabolically unhealthy will be equivalent to 1.2 with reference to be healthy, with the below described distribution of determinants and their expected Odds ratio, the minimal sample size would range from 757 to 780 on the one hand amongst normal weight individuals and on the other hand amongst overweight + obese subjects with an alpha of 0.05 and a power of 80%.

Covariate	Distribution	OR
Age	20-34y: 30%, 35-49y:30%, 50-64y:20%, 65-19y:10%, 80y et+:10%	1.2
Sex	Male:60%, Female:40%	1.3
Smoking	Not smoking: 50%, past smoker 25%, current smoker 25%	1.5
Alcohol	Not drinking: 47%, <1g/j:39%, 1-2g/j:8%, >2g/j:6%	0.6
Activities	0METs/j:36%, 1-49/j:16%, 50-131/j:16%, 132-279/j:16%, >279/j:16%	0.6
Waist C	normal m=96.4 sd=22.13	1.4

For a likelihood ratio Chi-square test of a single predictor, "Physical activity", which is distributed as ordinal((0, 0.33), (1, 0.33), (2, 0.33)), in logistic regression with a significance level of 0.05, in the presence of two covariates ("Diabetes" distributed as ordinal((0, 0.5), (1, 0.5)), "Obesity" distributed as ordinal((0, 0.5), (1, 0.5))), a sample size of 916 is required to obtain a power of at least 0.8 to detect a response odds ratio of 1.3 for a change of 1 in "Physical activity", assuming the response probability when all predictors are equal to their means is 0.25. The response odds ratios for the covariates are assumed to be 0.8 for a change of 1 in "Diabetes" and 0.8 for a change of 1 in "Obesity". The actual power is 0.8.

The objectives of the second wave of ORISCAV-LUX will be mainly to study the associations between continuous variables (for example: arterial stiffness as measured by the pulse wave velocity and the cognitive function, as measured by CANTAB). Assuming that we want to detect a correlation of 0.2 between two variables with a power of 90% and a type 1 error of 5%, the sample size needed would be 259.

9.4. Study limitations

Every study, no matter how well it is conducted and constructed, has limitations. The **possible** limitations that may affect or restrict our research analyses include:

1) This study aims to test several hypotheses with different endpoints (outcomes), therefore, failure to assess potential latent (unknown) confounding factors may exist.
2) Differences in the methodology of data collection, between the two waves. These differences concern principally the method of collection of self-reported data and the

new physical and clinical examinations added to the second wave. Therefore, a heavy training program should be planned to teach the research nurses on how to perform the new measurements, notably, the arterial stiffness, CANTAB, physical activity accelerometer and body composition.

3) A relatively long follow-up period of 6-7 years is a limitation for a number of research question, specifically those related to unstable lifestyle and dietary habits.
4) Although the FFQ questionnaires in this study showed adequate reliability, future research may consider the development of more reliable measures for examining dietary habits.
5) We should be cautious when presenting and interpreting the temporal tendency of cardiovascular risk factors, as the findings concern only two-point data collection, and consider the importance of successive waves to confirm the tendency.
6) Adequacy of the sample to effectively answer certain of the hypotheses/research questions. Despite the motivation and repeated contact, non-participation (drop-out) is still expected.

Certain statistical solutions are suggested à posteriori such as Benferroni correction, precision calculation and accept to publish null findings.

9.5. Non-participant data

A record of the participation status of each person invited to participate in the ORISCAV-LUX 2 will be kept. The number and type of contact attempts will also be recorded. If contact is made, the participation status (i.e. participates, refuses or drops out) will be recorded. Information on complete and incomplete examinations will also be recorded. If the person refuses to participate in any examination and the reason can be obtained, it will be recorded. Possible reasons for not being examined are listed below:

- Refused: no reason given
- Refused: lack of time
- Refused: personal principle
- Refused: health problem (e.g. disability restricting access to the examination site or they are hospitalised)
- Refused: feeling healthy (therefore thinks that there is no reason to participate)
- Refused: survey topic (is not interested in health issues or considers this too personal)
- Contacted: not able to schedule an appointment

- Contacted: no show (does not come to the scheduled visit, and the visit cannot be re-scheduled)
- Not contacted: not reached (no address/phone number available, outdated information)
- Not eligible: moved abroad, died.
- Other reason (should be specified, if feasible)

It is notable that sample representativeness will not be a major concern, as in the first wave of the ORISCAV-LUX.

9.6. Study outcomes variables

9.6.1. Primary outcomes variables

Main ORISCAV-LUX 2 indicators focus on the following diseases and risk factors which principally assessed as regards the First descriptive objectives.

- Blood pressure and hypertension
- Blood Lipids and cholesterol disorders
- Diabetes and glycaemic biomarkers
- Metabolically healthy/unhealthy Obesity, overweight and normal weight: Prevalence description
- Anthropometry Height, Weight, BMI, WHR, Hip C, and Proximal Thigh C. Visceral adipose tissue prediction: Descriptive analysis
- Total and segmental [right arm, left arm, trunk, right leg, and left leg] body composition [fat mass (FM), fat free mass (FFM) and predicted muscle mass (MM)]: Descriptive analysis
- Self-perceived health and other self-reported chronic diseases
- Lifestyle habits, including: smoking habits; alcohol consumption; dietary habits; and physical inactivity.

9.6.2. Secondary outcomes

Secondary outcomes will be principally assessed as regards the additional secondary analytic objectives. The following list presents the main secondary outcomes, among others, that will be assessed.

9.6.2.1. Anthropometry

Height, Weight, BMI, WHR, Hip Circumference, and Proximal Thigh Circumference. Visceral adipose tissue prediction: Analysis of the association with the metabolically healthy/unhealthy weight status.

9.6.2.2. Bio-impedance analysis

Total and segmental [right arm, left arm, trunk, right leg, and left leg] body composition [fat mass (FM), fat free mass (FFM) and predicted muscle mass (MM)]: Analysis of the association with the metabolically healthy/unhealthy weight status.

9.6.2.3. Metabolically healthy/unhealthy weight status

- Prevalence/incidence of the metabolically healthy/unhealthy normal weight, overweight and obesity between 2007 and 2015.
- Associations between the 6 metabolically healthy/unhealthy weight status phenotypes and their potential determinants [total fat and muscle masses, visceral adipose tissue, food habits, physical activity, sedentary lifestyle, pollutants]. Evolution between 2007 and 2015.
- Mean, min and max values of the body composition constituents amongst the weight status phenotypes [fat mass, muscle mass, visceral adipose tissue] of the metabolically healthy/unhealthy normal weight, overweight and obese phenotypes.
- Associations between the metabolically healthy/unhealthy weight status and the cognitive health / decline, respectively the cardiac functions and the healthy ageing / frailty.
- Associations between body composition [fat mass, muscle mass, visceral adipose tissue] and the cognitive health / decline, respectively the cardiac functions and the healthy ageing / frailty.

9.6.2.4. Cardiology

- Prevalence of ECG-measured cardiac disorders such as cardiac hypertrophy, atrial fibrillation, prolonged QT interval, and arrhythmias (%)
- Prevalence of self-reported cardiac diseases (%)
- Prevalence of cardiac treatments in the population (%)
- Prevalence of PWV (%)
- Associations between PWV and cognitive function.

- Association between traditional CVD risk factors, namely, hypertension, diabetes, smoking, alcohol consumption, lipid disorders and cognitive function

9.6.2.5. Renal function

- Mean and median creatinine clearance
- Prevalence of abnormal creatinine clearance (%)
- Prevalence of self-reported kidney disorders (%)

9.6.2.6. Cognitive function

- Estimate the prevalence of early cognitive impairment in Luxembourg
- Examine the association of diet with cognitive function

9.7. Statistical analysis

Statistical analyses will be performed using available statistical software, for example, SAS System version 9.3 (SAS Institute, Cary, NC, USA) or SPSS® for Windows Version 22.0. Results will be presented in tables and in graphics.

9.7.1. Univariate Analyses

The univariate analysis will describe independently each indicator. Qualitative variables will be described in terms of frequency (N), percentages (%) and missing data. 95% confidence intervals (CI) will be calculated. Quantitative variables will be described in terms of mean, median and standard error.

9.7.2. Bivariate Analyses

Qualitative variables will be compared using contingency tables. A Chi2 test will be applied for 2x2 tables. In case of theoretical frequencies lower than 5, the Fisher's exact test will be used. RxC tables will be analysed using the Fisher exact (Fisher-Freeman-Halton) test, independently of the obtained theoretical frequencies.

To evaluate the relationship between a continuous and a qualitative variable, the distribution of the continuous variables in accordance with the Gaussian law will be assessed in a first step

with the Kolmogorov-Smirnov test. In a second step, the Levene test will be used to control the homogeneity of variances. If the normality hypothesis is rejected, the Kruskall-Walis test will be performed. If the normality is respected but the homogeneity of variances hypothesis is rejected, the Welch test will be applied. If both, the normality and the homogeneity hypothesis are respected, an Analysis of Variance will be performed.

The association between two quantitative variables will be analysed using linear regression. The correlation coefficient will be indicated. All statistical tests will be two-sided with an alpha risk fixed at 5%. P-values will be given for all tests.

9.7.3. Multivariable Analyses

A logistic model will be used if response variable is binary. In case the response variable is quantitative, a multivariable regression will be accomplished. Odds ratios and their 95% CIs will be expressed. R, R2, and SEE (standard error estimation) will be calculated.

10. QUALITY CONTROL AND QUALITY ASSURANCE

10.1. L.I.H.'s quality assurance system

In the context of ORISCAV-LUX 2, the appropriate Standard Operating Procedures developed by the L.I.H. quality assurance unit will be applied. This study may also be subject to external quality assurance.

Similar to wave 1 of the ORISCAV-LUX study, strict control measures will be applied to ensure quality throughout the conduct of the study, namely, operational data-collection, data processing and reporting. These included: testing and validating the translated questionnaires; introducing multiple cross-checked questions on the same topic to validate the results; training of data collection nurses to perform their duties according to standard operating procedures; monitoring and ascertaining of instruments performance every morning before starting the tests on the subjects; daily assessment of paper forms for answer's relevance and completeness; designing the database in a way that allows to confirm the validity of the participant's identification codes, establishing the completeness of the entered data and performing basic data checks; training of data processing personnel to be able to provide reliable and accurate documentation, as well as periodic summary reports on the latest project progress; independent double data entry followed by matching and checking for data-entry errors, data cleaning according to experts' consensus; finally, checking of the internal and external consistency of the analyzed data before reporting.

10.2. Training programme

All survey team members such as study nurses, laboratory technicians, administrative assistants, data manager, statistician and IT specialist will be appropriately trained in the following topics.

- General presentation of the survey
- Survey organisation, roles and responsibilities
- Confidentiality and informed consent procedures
- Management of participants visits
- Selected measurements
- Data management and IT skills

- Standardisation and quality assurance

10.3. Quality control and assessment

10.3.1. Internal quality control and assessment

10.3.1.1. Day to day or weekly quality control checks

The day-to-day quality control checks for the survey measurement will be performed by the fieldwork team.

10.3.1.2. Monitoring visits

Quality control will also be performed through regular internal monitoring visits to the examination sites. Internal audit visits will be done periodically by L.I.H. assigned team members to ensure that all aspects of the survey protocol and procedures are followed and that applicable regulations and obligations are being fulfilled. Source documents will be reviewed for verification of consistency with data on CRFs. The corresponding source documents for survey participant will be made available provided that subject confidentiality is maintained in accord with local regulations (162).

10.3.2. External quality control assessment

The study may also be subject to external quality assurance audits and/or inspections by the ethic committee, and the national commission for data protection (CNPD). It is important that the relevant staff members are available for any audits and/or inspections.

10.4. Risk management

Similar to the first wave of the ORISCAV-LUX study, strict control measures will be applied to ensure quality throughout the steps of the second wave, namely, sample selection, operational data-collection, data processing and reporting. The following table represents an analysis of potential risks across different study's stages and suggested solutions.

Table 2 Risk analyses and suggested solutions

Study's stages	Potential risk	Suggested solutions
Project elaboration and preparation	Insufficient expertise for protocol writing, survey design	Requesting the support and validation of the Head of the unit (Dr. Saverio Stranges) for decision-making
	Insufficient personnel for planning and preparation	The PI is supported by a group of researchers to write the scientific part of the project, by a Coordinator nurse for the Fieldwork preparation, and by a Project Manager for global organisation
	Ethical approval delayed which may lead to delay in pilot and real phases	Careful preparation of the research protocol and survey documents. Ensure that the needed resources are available
Field work	Understaffed project team	A realistic project plan will be scheduled to co-ordinate and execute the work packages. Projects needs have been analysed and discussed with the Direction to recruit sufficient resources
	Lower participation rate than expected, which may influence the sample size and study precision	Several measures will be organized to motivate the participants, e.g., Media campaigns, careful planning of the recruitment process, personnel availability to answer participant's questions,
	Logistic problems, e.g., Internet interruption, equipment breakdown.	An alternative Paper versions of questionnaire will be easily available in the investigation centres. An in-house IT support is always available
	Risks of violence or harm caused to staff members or participants during fieldwork	The management of situations with aggressive and violent participants or other safety risks during fieldwork is explained in the study protocol and will be discussed in the training session. In addition, there will be a supervision of field work staff throughout the fieldwork process.
Dataset management	Violation of personal data protection rules	Careful planning and preparation for data management and proper training for all survey staff will allow to avoid such risk
Data analysis	Lack of technical know-how in dataset and statistical analysis	Nicolas Sauvageot (bio-statistician), who has worked with the PI and has accumulated experience from the previous research work on the first wave of ORISCAV-LUX project, is allocated to work on this project

	Sample size too small, not enough power to see trends among subgroups	Study power and precision calculation has been done to answer main research questions. Alternative statistical solution would be available, for example, Benferroni correction, and accept to publish null findings
Project management	Overshooting of timelines or planned targets	Project delays can be avoided by the use of an in-house "project follow-up" monitoring system. This system maintains accurate documentation of all inputs and outputs all over the life of the project. The goal of this system is to track project progress to ensure that the project is on track with scheduled deliverables
	Crossing the stipulated budget	A budget control system for all L.I.H. projects is already operating, with internal support from the office of Accountability service
	Delay in recruitment of adequate staff (research nurse)	The recruitment procedure will be started with the help of Human resources service, as soon as possible
	Unmotivated staff	The global scientific environment within the L.I.H., as well as the primordial role of the PI, Direction supervision, and Project management support will help to motivate and increase their interest to research and hence diminish this risk

11. ETHICAL CONSIDERATIONS

The Principal and medical investigators will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki (165) and the deontology code in epidemiology(166).

11.1. Ethics and data protection

The research protocol and the associated informed consent documents is designed in accordance with the Luxembourgish legislation and regulations (Law relative to the protection of the individual towards personal data processing *("Loi relative à la protection des personnes à l'égard du traitement des données à caractère personnel*"), Grand Ducal Law of the 27th of July, 2007. In compliance with this law, the research protocol must be submitted to the national ethics committee (CNER) and notified to the national commission for data protection (CNPD).

Any amendments to the protocol or consent materials must also be approved before they are placed into use.

11.2. Informed Consent Process

The selected subjects will receive an invitation letter together with notice of information sent by mail to their home. L.I.H. contact phone number and e-mail address will be provided in order to answer any questions the participant may have. There will also be a media campaign throughout the country, mainly through newspapers and flyers to raise awareness.

The nurse will check if the participant understood the provided information and has the adequate decision capacity. The study nurse will remind the participant of the following: she/he is free to choose if she/he wants to participate, she/he has the right to withdraw from the study whenever she/he wants and she/he can refuse any measurement at any time. The participant must understand the objective of the survey, the uses of the data and the measures taken to ensure data confidentiality.

The informed consent form will be filled out in duplicate. The first exemplar will be kept under the responsibility of the medical investigator and the second exemplar will be given to the survey participant.

In case of choosing to fill in the auto-administered questionnaire on-line, there will be a box to be firstly ticked by the participant to indicate his/her consent to participate.

11.3. Subject Confidentiality

The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the survey or the data will be released to any unauthorised third party without prior written approval of the L.I.H.

Participants will be identified on the questionnaires and CRFs by a research participant number. A separate identification will be kept that matches identifying codes with the participant's name and residence. Other not anonymous, identifiable paper or electronic source documents such as the signed informed consent forms, the medical feedback letter and the appointment books will be kept in a secured place under the responsibility of the medical and principal investigators (162).

The medical and principal investigators have the responsibility to keep all study documents on file for at least 10 years after completion or discontinuation of the study.

12. FINANCES AND INSURANCE

12.1. Financial remuneration

Participants will not receive any payment for participation. The transport will not be reimbursed.

12.2. Insurance

The L.I.H. will subscribe an insurance before the launch of the study. It will be insured to indemnify the medical investigator against any claim for damages brought by a participant who suffers from a research related injury that happened on the examination site or on the way to it.

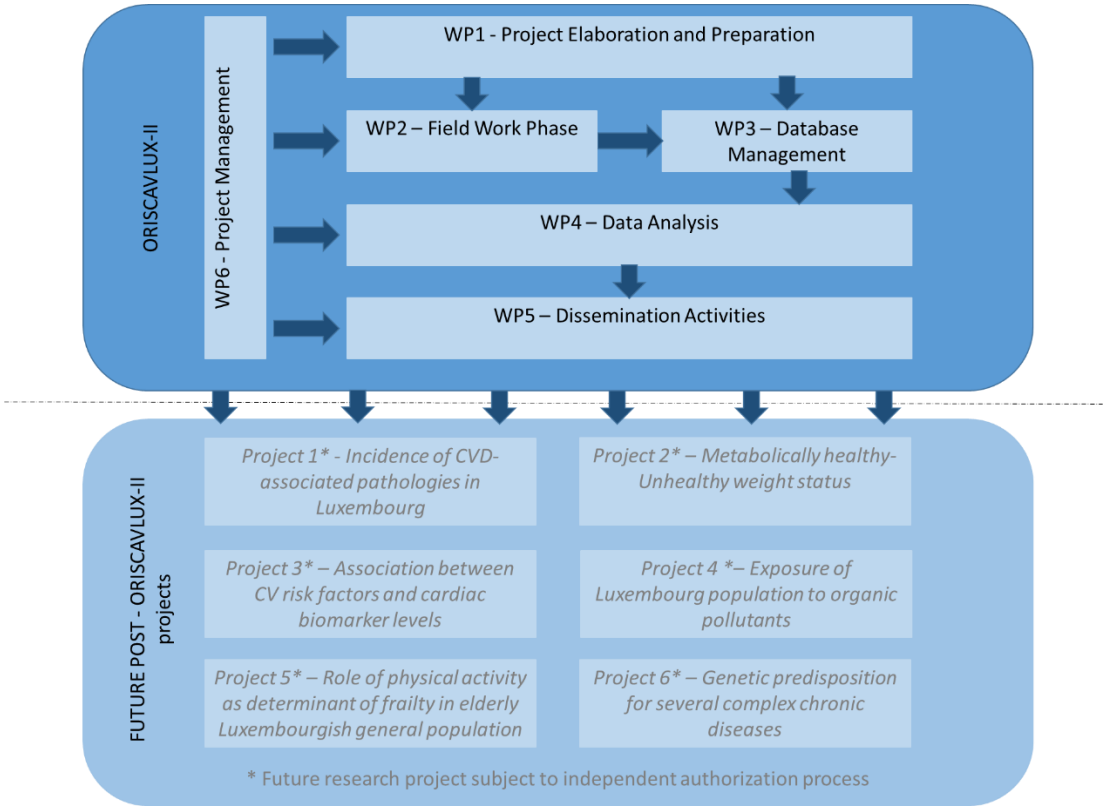
13. PROJECT WORK PLAN

This project is planned to be split into six work-packages (WP) with a lifetime of 36 months, from 01/01/2015 to 31/12/2017, as seen in Figure 5:

- WP1: Project elaboration and preparation
- WP2: Field work
- WP3: Database management
- WP4: Data analysis
- WP5: Dissemination activities
- WP6: Project management

Several subsequent research projects are foreseen to be developed after 2017 to analyse further scientific questions. These subsequent projects will be subject to separate funding and ethical bodies' authorizations.

Figure 7 Design of the ORISCAV-LUX 2 project plan



WP number	**1**		
WP title	Project elaboration and preparation		
WP leader	Ala'a Al Kerwi (Principal Investigator)		
Start date	01/01/2015	End date	30/04/2015
Objective			
WP1 consists of developing both the project protocol and the administrative files such as the ethical committee file, budget, and funding authorization form. It includes also the development of the questionnaire content as well as the set of examinations and the biological sample's needs. This work package will be achieved in collaboration with Coordinator nurse, Project manager and the other researchers interested by the ORISCAV-LUX 2 study.			
Tasks			
T1.1 Definition of scientific questions and interests T1.2 Development of questionnaire content T1.3 Development and selection of examination tests and equipment T1.4 Definition of biological samples needs, in terms of volume and type of analysis T1.5 Project protocol elaboration T1.6 Budget elaboration T1.7 Preparation of LIH Management authorization file and form T1.8 Preparation and submission of the CNER file, and the CNPD notification and insurance contract			
Interdependence with other work packages			
WP1 is the basis to build up the other WPs.			
Deliverables and milestones			
<u>Deliverables:</u> D1.1: Project protocol D1.2: CNER file, including participant questionnaire, examination and biological sample contents			

D1.3: CNPD notification D1.4: Project budget D1.5: LIH Management file Milestones: MS1: CNER advice MS2: L.I.H. Management authorization

Human resources			
Name of researcher	Initials	Qualification level	Person*months
Alaa Al Kerwi	AAK	Senior Researcher	2
Marylène d'Incau	MDI	Fieldwork Coordinator	2
Dominique Mormont	DM	Project Manager	1
Graziella Ambroset	GAM	Research Nurse	2
Melanie Kiemen	MK	Research Nurse	2
Agnès Columeau	ACO	Research Nurse	1,6
Jessica Pastore	JP	Data Manager	1
Maxime Larcelet	ML	IT Specialist	1,2
Nicolas Sauvageot	NS	Bio-Statistician	1

WP number	**2**		
WP title	Field Work Phase		
WP leader	Marylène d'Incau (fieldwork coordinator)		
Start date	01/04/2015	End date	30/09/2016
Objective			
WP2 aims to prepare, set up and manage the recruitment of the participants			

Tasks
T2.1: Training of research nurses to perform their duties according to standard operating procedures T2.2: Performing of pilot phase T2.3: Recruitment of participants T2.4: Implementation of Field work, including interviewing of the participant, examination and biological samples collection T2.5 : Return a feedback to the participant T2.6: Achievement of full data collection
Interdependence with other work packages
WP2 is set up based on WP1 and is the data source for WP3
Deliverables and milestones: ORISCAV-LUX 2 dataset is constituted
Deliverables: D2.1: Research nurse training module D2.2: Evaluation of pilot phase and possible adaptation of recruitment procedure D2.3: Information letter and consent form, self-reported questionnaire, nurse interview questionnaire, examinations and data for analyses of biological samples Milestones: MS 3: Participants' recruitment phase is completed
Human resources

Name of researcher	Initials	Qualification level	Person*months
Marylène d'Incau	MDI	Field work coordinator	16
Graziella Ambroset	GA	Research nurse	16
Agnès Columeau	AC	Research nurse	12,8
Melanie Kiemen	MK	Research nurse	16
Research Nurse	(to be defined)	Research nurse	16,5
Research Nurse (P)	(to be defined)	Research nurse	4,4
Cathy Fousse	CF	Secretary	12,6
Maxime Larcelet	ML	IT Specialist	3,2
Ala'a Alkerwi	AAK	Project leader (PI)	0,5

Summer students	Stud	Summer students	7 x 1

WP number	**3**		
WP title	Database Management		
WP leader	Ala'a Al Kerwi (PI)		
Start date	01/04/2015	End date	31/12/2016
Objective			
WP3 aims to collect and manage data obtained from participant recruitment			
Tasks			
T3.1: Preparation of data entry form T3.2: Setup of database T3.3: Double independent data entry T3.4: Matching and checking for data-entry errors T3.5: Database cleaning and quality control measures			
Interdependence with other work packages			
WP3 directly depends on the progress of WP2 and is a necessary basis to achieve WP4.			

Deliverables and milestones
Deliverables: D3.1: Data entry form D3.2: Complete data set Milestones: ORISCAV-LUX 2 dataset is frozen and ready for the analyses

Human resources			
Name of researcher	Initials	Qualification level	Person*months
Jessica Pastore Nicolas Sauvageot Alaa Al Kerwi Marylène d'Incau Agnès Columeau Graziella Ambroset Melanie Kiemen	JP NS AAK MDI ACO GAM MK	Data manager Bio-statistician Project leader (PI) Field work coordinator Research nurse Research nurse Research nurse	9 1 1 2 2,4 3 3

WP number	**4**		
WP title	Data analysis		
WP leader	Ala'a Al Kerwi (PI)		
Start date	01/10/2016	End date	30/06/2017
Objective			
WP4 aims to explore the collected dataset in order to provide a descriptive analysis of the second wave dataset as well as a description of temporal evolution of classical potentially modifiable cardiovascular risk factors between wave 1 and wave 1l. Specifically 1) Assessment of major traditional and emerging cardiovascular risk factors and associated			

conditions, such as diabetes, hypertension, lipid disorders, and 2) Description of temporal evolution of classical potentially modifiable cardiovascular risk factors between 2008 and 2016
Tasks
T4.1: Dataset exploration and descriptive analysis T4.2: Writing technical report on dataset exploration T4.3: Literature review, T4.4: Statistical dataset analyses, T4.5: Data interpretation, T4.6: Reporting.
Interdependence with other work packages
WP4 is directly linked to the achievement of WPs 2 and 3 and should lead to perform WP5
Deliverables and milestones
Deliverables: D4.1: Report on descriptive analysis of the ORISCAV-LUX 2 dataset D4.2: Draft of a scientific peer-reviewed article (temporal evolution of classical risk factors) D4.3: Draft of « Enjeux santé » (French publication) Milestones: The analyses is accomplished
Human resources

Name of researcher	Initials	Qualification level	Person*months
Alaa Al Kerwi Nicolas Sauvageot Jessica Pastore Etudiant Master	AAK NS JP Mas1	Project leader (PI) Biostatistician Data manager Master student (1)	3 8 4 5

WP number	**5**		
WP title	Dissemination activities		
WP leader	Alaa Al Kerwi		
Start date	01/07/2017	End date	31/12/2017
Objective			
WP5 aims to disseminate outputs of the ORISCAV-LUX 2 project to scientific national and international community as well as to large Luxembourgish public			
Tasks			
T5.1: Organization of oral presentations to public health authorities and healthcare professionals T5.2: Organization of oral presentations in in–house scientific staff meetings T5.3: Organization of a press conference T5.4 : Article submission and tracking publication process T5.5: Production of a new issue of “Enjeux Santé”			
Interdependence with other work packages			
WP5 is the direct consecutive step following WP4 data analysis.			
Deliverables and milestones			
Deliverables D5.1: Oral presentations to public health authorities, healthcare professionals D5.2: Oral presentation in in-house scientific staff session D5.3: Dissemination of the findings in Enjeux Santé brochure D5.4: Submission of an abstract to an international conference			

Milestones			
Human resources			
Name of researcher	Initials	Qualification level	Person*months
Ala'a Al Kerwi Master student	AAK Mas2	Project leader (PI) Master student (2)	5 5

WP number	**6**		
WP title	Project management		
WP leader	Alaa Al Kerwi		
Start date	01/01/2015	End date	31/12/2017
Objective			
With the support of project manager, project assistant and coordinator nurse, the PI will accomplish WP6 which aims to manage the different administrative and financial aspects of the project and to coordinate the work within the project staff. It also aims to ensure communication to internal and external stakeholders.			
Tasks			
T6.1: Project controlling and monitoring T6.2: Organization and management of regular team meetings T6.3: Regular communication of the project status to the different stakeholders T6.4: Writing intermediate and final activity reports.			
Interdependence with other work packages			
WP6 is interconnected to all the other WPs.			

Deliverables and milestones			
Deliverables: D6.1: Intermediate scientific and financial report 1 (31/01/2016) D6.2: Intermediate scientific and financial report 2 (31/01/2017) D6.3: Final report (31/01/2018) D6.4: Minutes of the different team meetings, which will be organized at regular basis (one meeting/month) D6.5: Regular communication of the project status to the different stakeholders			
Human resources			
Name of researcher	Initials	Qualification level	Person*months
Ala'a Al Kerwi Dominique Mormont Marylène d'Incau Manon Gantenbein Anna Chioti	AAK DM MDI MG AC	Project leader (PI) Project manager Coordinator nurse Ass. Head of Unit Head of Unit	1,5 1 1 0,6 0,6

14. PROJECT VALORIZATION

Type of valorization (pls mark your choices with "X" in the right column)	Primary valorisation	Secondary valorisation
Patent filing for new inventions	-	-
Publications in peer reviewed journals	X	X XX
Poster presentations, invited talks etc.	X	X XX
PhD training	-	X X
Master student training	X	XX
Competitive proposal submission (e.g; FNR, H2020…)	X	X XX
Reinforce the international collaboration	X	X XX
New methods and/or procedures	X	X XX
Database or registry	X	X XX
National Publications and Presentations	X	X XX
Other		

Here-after, we present several examples of activities to disseminate to post-ORISCAV-LUX 2 projects:

Publications in peer reviewed journals or magazines, 2015

1. **The metabolically healthy/unhealthy weight status. Prevalence, incidence, determinants and associated comorbidities**

- Metabolically unhealthy people in Luxembourg: Should we be worried? (ORISCAV I data). *BioMed Research International Special Issue on "Obesity, Diabetes, and Cardiometabolic Syndrome".*

+/- Enjeux Santé +/- Local Press. Submission deadline: post scientific publication.

- Metabolically healthy/unhealthy weight status in Luxembourg: environmental and behaviour determinants. (ORISCAV I data). *Obesity.*

+/- Enjeux Santé +/- Local Press. Submission deadline: post scientific publication.

Post ORISCAV II data collection –2016-

Scientific publications +/- Enjeux Santé +/- Local Press.

- Prevalence / incidence of the metabolically healthy/unhealthy normal weight, overweight and obesity (2007-2015). (ORISCAV I-II data). *BMC*
- The transition between the metabolically healthy/unhealthy weight status phenotypes (2007-2015) and the associated causes/determinants. (ORISCAV I-II data). *Obesity.*
- Body composition characteristics of the metabolically healthy/unhealthy normal weight, overweight and obese phenotypes. (ORISCAV II data). *Obesity.*
- Associations between metabolically healthy/unhealthy weight status and cognitive decline. *Obesity.*
- Associations between metabolically healthy/unhealthy weight status, traditional and new emergent CV risk factors. *Obesity.*
- Associations between metabolically healthy/unhealthy weight status and healthy ageing/frailty. *Obesity.*
- Associations between body composition and cognitive decline. *Obesity.*
- Associations between body composition, traditional and new emergent CV risk factors. *Obesity.*
- Associations between body composition and healthy ageing / frailty.

Poster presentations, invited talks etc.

ECO2016, the 23nd European Congress on Obesity

ICO2016, the XIII International Conference on Obesity

ECO2017, the 24nd European Congress on Obesity

Journées européennes de l'obésité, May 2015-2016-2017.

2. **The role of physical activity as determinant of frailty in elderly Luxembourgish general population**
    - The first article will clarify whether the objective of the intensity of physical activity has a strong association with physical function than the other dimensions of physical activity. American Journal of Public Health, impact factor 3.9.
    - The second article will be based on the association between fitness level and physical activity measured with objective methods and physical functioning. Journal of Sports Sciences, impact factor 2.08.
    - The third article will focus on sedentary behaviour at baseline and physical functioning at follow-up. Ageing, Impact factor 3.8.

15. PUBLICATION POLICY

It is anticipated that the results of this survey will be published in national and international reports. Findings of medical importance will be published in international peer-reviewed journals and/or presented at scientific meetings. Authorship will be determined by mutual agreement between the different parties involved.

Before publication, all study results are considered confidential and shall not be made available to any third party by any member of the investigating team without an appropriate confidentiality agreement.

16. BIBLIOGRAPHY

1. Mendis S, Puska P, Norrving, editors. B. Global Atlas on Cardiovascular Disease Prevention and Control. World Health Organization, Geneva. 2011.
2. Brundtland GH. From the World Health Organization. Reducing risks to health, promoting healthy life. Jama. 2002;288(16):1974.
3. Murray CJ, Lopez AD. Evidence-based health policy--lessons from the Global Burden of Disease Study. Science. 1996;274(5288):740-3.
4. O'Donnell CJ, Elosua R. [Cardiovascular risk factors. Insights from Framingham Heart Study]. Revista espanola de cardiologia. 2008;61(3):299-310.
5. Greenland P, Gidding SS, Tracy RP. Commentary: lifelong prevention of atherosclerosis: the critical importance of major risk factor exposures. Int J Epidemiol. 2002;31(6):1129-34.
6. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet. 2004;364(9438):937-52.
7. Criqui MH, Barrett-Connor E, Holdbrook MJ, Austin M, Turner JD. Clustering of cardiovascular disease risk factors. Prev Med. 1980;9(4):525-33.
8. Smoak CG, Burke GL, Webber LS, Harsha DW, Srinivasan SR, Berenson GS. Relation of obesity to clustering of cardiovascular disease risk factors in children and young adults. The Bogalusa Heart Study. Am J Epidemiol. 1987;125(3):364-72.
9. Chu NF, Rimm EB, Wang DJ, Liou HS, Shieh SM. Clustering of cardiovascular disease risk factors among obese schoolchildren: the Taipei Children Heart Study. The American journal of clinical nutrition. 1998;67(6):1141-6.
10. Engstrom G, Jerntorp I, Pessah-Rasmussen H, Hedblad B, Berglund G, Janzon L. Geographic distribution of stroke incidence within an urban population: relations to socioeconomic circumstances and prevalence of cardiovascular risk factors. Stroke. 2001;32(5):1098-103.
11. Keys A. Alpha lipoprotein (HDL) cholesterol in the serum and the risk of coronary heart disease and death. Lancet. 1980;2(8195 pt 1):603-6.
12. Kannel WB, Castelli WP, McNamara PM, McKee PA, Feinleib M. Role of blood pressure in the development of congestive heart failure. The Framingham study. N Engl J Med. 1972;287(16):781-7.

13. MacMahon S, Peto R, Cutler J, Collins R, Sorlie P, Neaton J, et al. Blood pressure, stroke, and coronary heart disease. Part 1, Prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. Lancet. 1990;335(8692):765-74.
14. Savage PJ. Treatment of diabetes mellitus to reduce its chronic cardiovascular complications. Curr Opin Cardiol. 1998;13(2):131-8.
15. Manson JE, Colditz GA, Stampfer MJ, Willett WC, Rosner B, Monson RR, et al. A prospective study of obesity and risk of coronary heart disease in women. N Engl J Med. 1990;322(13):882-9.
16. Criqui MH, Cowan LD, Tyroler HA, Bangdiwala S, Heiss G, Wallace RB, et al. Lipoproteins as mediators for the effects of alcohol consumption and cigarette smoking on cardiovascular mortality: results form the Lipid Research Clinics Follow-up Study. Am J Epidemiol. 1987;126(4):629-37.
17. Hjermann I, Velve Byre K, Holme I, Leren P. Effect of diet and smoking intervention on the incidence of coronary heart disease. Report from the Oslo Study Group of a randomised trial in healthy men. Lancet. 1981;2(8259):1303-10.
18. Ornish D, Brown SE, Scherwitz LW, Billings JH, Armstrong WT, Ports TA, et al. Can lifestyle changes reverse coronary heart disease? The Lifestyle Heart Trial. Lancet. 1990;336(8708):129-33.
19. Murray CJ, Lopez AD. Measuring the global burden of disease. The New England journal of medicine. 2013;369(5):448-57.
20. Pardo Silva MC, De Laet C, Nusselder WJ, Mamun AA, Peeters A. Adult obesity and number of years lived with and without cardiovascular disease. Obesity. 2006;14(7):1264-73.
21. Guh DP, Zhang W, Bansback N, Amarsi Z, Birmingham CL, Anis AH. The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis. BMC public health. 2009;9:88.
22. Jaarsma T, Deaton C, Fitzsimmons D, Fridlund B, Hardig BM, Mahrer-Imhof R, et al. Research in cardiovascular care: a position statement of the Council on Cardiovascular Nursing and Allied Professionals of the European Society of Cardiology. European journal of cardiovascular nursing : journal of the Working Group on Cardiovascular Nursing of the European Society of Cardiology. 2014;13(1):9-21.
23. Lloyd-Jones DM. Cardiovascular risk prediction: basic concepts, current status, and future directions. Circulation. 2010;121(15):1768-77.
24. Tzoulaki I, Liberopoulos G, Ioannidis JP. Assessment of claims of improved prediction beyond the Framingham risk score. JAMA. 2009;302(21):2345-52.

25. Hackam DG, Anand SS. Emerging risk factors for atherosclerotic vascular disease: a critical review of the evidence. JAMA. 2003;290(7):932-40.
26. Miller MA, Cappuccio FP. Biomarkers of cardiovascular risk in sleep-deprived people. Journal of human hypertension. 2013;27(10):583-8.
27. Schwartz J. Air pollution and blood markers of cardiovascular risk. Environmental health perspectives. 2001;109 Suppl 3:405-9.
28. The IDF consensus worldwide definition of the metabolic syndrome. International Diabetes Federation. Available from: wwwidforg/webdata/docs/IDF_metasyndrome_definitionpdf. 2005.
29. Pede S, Lombardo M. [Cardiovascular risk stratification. Systolic, diastolic or pulse pressure?]. Ital Heart J Suppl. 2001;2(4):356-8.
30. Liao J, Farmer J. Arterial stiffness as a risk factor for coronary artery disease. Current atherosclerosis reports. 2014;16(2):387.
31. Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, et al. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Hypertension. 2001;37(5):1236-41.
32. Payne RA, Wilkinson IB, Webb DJ. Arterial stiffness and hypertension: emerging concepts. Hypertension. 2010;55(1):9-14.
33. Jatoi NA, Jerrard-Dunne P, Feely J, Mahmud A. Impact of smoking and smoking cessation on arterial stiffness and aortic wave reflection in hypertension. Hypertension. 2007;49(5):981-5.
34. Orr JS, Gentile CL, Davy BM, Davy KP. Large artery stiffening with weight gain in humans: role of visceral fat accumulation. Hypertension. 2008;51(6):1519-24.
35. Ghiadoni L, Penno G, Giannarelli C, Plantinga Y, Bernardini M, Pucci L, et al. Metabolic syndrome and vascular alterations in normotensive subjects at risk of diabetes mellitus. Hypertension. 2008;51(2):440-5.
36. European Society of Hypertension-European Society of Cardiology Guidelines C. 2003 European Society of Hypertension-European Society of Cardiology guidelines for the management of arterial hypertension. J Hypertens. 2003;21(6):1011-53.
37. Palatini P, Casiglia E, Gasowski J, Gluszek J, Jankowski P, Narkiewicz K, et al. Arterial stiffness, central hemodynamics, and cardiovascular risk in hypertension. Vasc Health Risk Manag. 2011;7:725-39.
38. Niedziela J, Hudzik B, Niedziela N, Gasior M, Gierlotka M, Wasilewski J, et al. The obesity paradox in acute coronary syndrome: a meta-analysis. European journal of epidemiology. 2014;29(11):801-12.

39. Meigs JB, Wilson PW, Fox CS, Vasan RS, Nathan DM, Sullivan LM, et al. Body mass index, metabolic syndrome, and risk of type 2 diabetes or cardiovascular disease. The Journal of clinical endocrinology and metabolism. 2006;91(8):2906-12.
40. Bo S, Musso G, Gambino R, Villois P, Gentile L, Durazzo M, et al. Prognostic implications for insulin-sensitive and insulin-resistant normal-weight and obese individuals from a population-based cohort. The American journal of clinical nutrition. 2012;96(5):962-9.
41. Shea JL, Randell EW, Sun G. The prevalence of metabolically healthy obese subjects defined by BMI and dual-energy X-ray absorptiometry. Obesity. 2011;19(3):624-30.
42. Ogorodnikova AD, Kim M, McGinn AP, Muntner P, Khan U, Wildman RP. Incident cardiovascular disease events in metabolically benign obese individuals. Obesity. 2012;20(3):651-9.
43. Wildman RP, Muntner P, Reynolds K, McGinn AP, Rajpathak S, Wylie-Rosett J, et al. The obese without cardiometabolic risk factor clustering and the normal weight with cardiometabolic risk factor clustering: prevalence and correlates of 2 phenotypes among the US population (NHANES 1999-2004). Archives of internal medicine. 2008;168(15):1617-24.
44. Hamer M, Stamatakis E. Metabolically healthy obesity and risk of all-cause and cardiovascular disease mortality. The Journal of clinical endocrinology and metabolism. 2012;97(7):2482-8.
45. Camhi SM, Waring ME, Sisson SB, Hayman LL, Must A. Physical activity and screen time in metabolically healthy obese phenotypes in adolescents and adults. Journal of obesity. 2013;2013:984613.
46. Sims EA. Are there persons who are obese, but metabolically healthy? Metabolism: clinical and experimental. 2001;50(12):1499-504.
47. Karelis AD. Metabolically healthy but obese individuals. Lancet. 2008;372(9646):1281-3.
48. Bluher S, Schwarz P. Metabolically healthy obesity from childhood to adulthood - Does weight status alone matter? Metabolism: clinical and experimental. 2014;63(9):1084-92.
49. Yoo HJ, Hwang SY, Hong HC, Choi HY, Seo JA, Kim SG, et al. Association of metabolically abnormal but normal weight (MANW) and metabolically healthy but obese (MHO) individuals with arterial stiffness and carotid atherosclerosis. Atherosclerosis. 2014;234(1):218-23.
50. Perez-Martinez P, Alcala-Diaz JF, Delgado-Lista J, Garcia-Rios A, Gomez-Delgado F, Marin-Hinojosa C, et al. Metabolic phenotypes of obesity influence triglyceride and inflammation homoeostasis. European journal of clinical investigation. 2014;44(11):1053-64.

51. Ferrannini E, Natali A, Bell P, Cavallo-Perin P, Lalic N, Mingrone G. Insulin resistance and hypersecretion in obesity. European Group for the Study of Insulin Resistance (EGIR). The Journal of clinical investigation. 1997;100(5):1166-73.
52. Green AK, Jacques PF, Rogers G, Fox CS, Meigs JB, McKeown NM. Sugar-sweetened beverages and prevalence of the metabolically abnormal phenotype in the Framingham Heart Study. Obesity. 2014;22(5):E157-63.
53. Lee K. Metabolically obese but normal weight (MONW) and metabolically healthy but obese (MHO) phenotypes in Koreans: characteristics and health behaviors. Asia Pacific journal of clinical nutrition. 2009;18(2):280-4.
54. Gauthier MS, Rabasa-Lhoret R, Prud'homme D, Karelis AD, Geng D, van Bavel B, et al. The metabolically healthy but obese phenotype is associated with lower plasma levels of persistent organic pollutants as compared to the metabolically abnormal obese phenotype. The Journal of clinical endocrinology and metabolism. 2014;99(6):E1061-6.
55. Pestana D, Faria G, Sa C, Fernandes VC, Teixeira D, Norberto S, et al. Persistent organic pollutant levels in human visceral and subcutaneous adipose tissue in obese individuals--depot differences and dysmetabolism implications. Environmental research. 2014;133:170-7.
56. Kim KS, Lee YM, Kim SG, Lee IK, Lee HJ, Kim JH, et al. Associations of organochlorine pesticides and polychlorinated biphenyls in visceral vs. subcutaneous adipose tissue with type 2 diabetes and insulin resistance. Chemosphere. 2014;94:151-7.
57. Kuk JL, Katzmarzyk PT, Nichaman MZ, Church TS, Blair SN, Ross R. Visceral fat is an independent predictor of all-cause mortality in men. Obesity. 2006;14(2):336-41.
58. Jensen MD. Role of body fat distribution and the metabolic complications of obesity. The Journal of clinical endocrinology and metabolism. 2008;93(11 Suppl 1):S57-63.
59. Dominguez LJ, Barbagallo M. The cardiometabolic syndrome and sarcopenic obesity in older persons. Journal of the cardiometabolic syndrome. 2007;2(3):183-9.
60. Peppa M, Koliaki C, Papaefstathiou A, Garoflos E, Katsilambros N, Raptis SA, et al. Body composition determinants of metabolic phenotypes of obesity in nonobese and obese postmenopausal women. Obesity. 2013;21(9):1807-14.
61. Camhi SM, Katzmarzyk PT. Differences in body composition between metabolically healthy obese and metabolically abnormal obese adults. International journal of obesity. 2014;38(8):1142-5.
62. Kim TN, Park MS, Yang SJ, Yoo HJ, Kang HJ, Song W, et al. Body size phenotypes and low muscle mass: the Korean sarcopenic obesity study (KSOS). The Journal of clinical endocrinology and metabolism. 2013;98(2):811-7.

63. Rappaport SM. Discovering environmental causes of disease. J Epidemiol Community Health. 2012;66:99-102.
64. Hiemminki K, Czene K. Attributable risks of familial cancer from the family-cancer database. Cancer Epidemiol Biomark Prev. 2002;11:1638-44.
65. Marenberg ME, Risch N, Berkman LF, Floderus B, de Faire U. Genetic susceptibility to death from coronary heart disease in a study of twins. N EnglJ Med. 1994;330:1041-6.
66. Vineis P, Khan AE, Vlaanderen J, Vermeulen R. The impact of new research technologies on our understanding of environmental causes of disease: the concept of clinical vulnerability. Environ Health 2009;8:54-67.
67. Boffetta P, McLaughlin JK, la Vecchio C, Autier P, Boyle P. "Environment" in cancer causation and etiological fraction: limitations and ambiguities. Carcinogenesis. 2007;28(5):913-5.
68. Stewart BW. Priorities for cancer prevention: lifestyle choices versus unavoidable exposures. Lancet. 2012;13:e126-e33.
69. Thayer KA, Heindel J, Bucher J, Gallo M. Role of Environmental Chemicals in Diabetes and Obesity: A National Toxicology Program Workshop Review. Environ Health Perspec. 2012;120:779-89.
70. Karoutsou E, Polymeris A. Environmental endocrine disruptors and obesity. Endocr Regul. 2012;46:37-46.
71. Newbold RR, Padilla-Banks E, Jefferson WN. Environmental estrogens and obesity. MolecCellEndocrinol. 2009;304:84-9.
72. Polyzos SA, Kountouras J, Deretzi G, Zavos C, Mantzoros CS. The emerging role of endocrine disruptors in pathogenesis of insulin resistance: a concept implicating nonalcoholic fatty liver disease. CurrMolecMed. 2012;12:68-82.
73. Porta M. Persistent organic pollutants and the burden of diabetes. Lancet. 2006;368:558-9.
74. Ruzzin J. Public health concern behind the exposure to persistent organic pollutants and the risk of metabolic diseases. BMC Public Health. 2012;12:298.
75. Ruzzin J, Petersen R, Meugnier E, Madsen L, Lock E, Lillefosse H, et al. Persistent Organic Pollutant Exposure Leads to Insulin Resistance Syndrome. Environ Health Perspec. 2010;118:465-71.
76. Arzuaga X, Ren N, Stromberg A, Black E, Arsenescu V, Cassis L, et al. Induction of gene pattern changes associated with dysfunctional lipid metabolism induced by dietary fat and exposure to a persistent organic pollutant. Toxicol Lett. 2009;189:96-101.

77. Irigaray P, Ogier V, Jacquenet S, Notet V, Sibille P, Méjean L, et al. Benzo[a]pyrene impairs b-adrenergic stimulation of adipose tissue lipolysis and causes weight gain in mice - A novel molecular mechanism of toxicity for a common food pollutant. FASEB J. 2006;273:1362-72.

78. Bhaskar R, Mohanty B. Pesticides in mixture disrupt metabolic regulation: In silico and in vivo analysis of cumulative toxicity of mancozeb and imidacloprid on body weight of mice. GenComparEndocrinol. 2014;205:226-34.

79. Marmugi A, Lasserre F, Beuzelin D, Ducheix S, Huc L, Polizzi A, et al. Adverse effects of long-term exposure to bisphenol A during adulthood leading to hyperglycaemia and hypercholesterolemia in mice. Toxicology. 2014;325:133-43.

80. Forbes LJL, Patel MD, Rudnicka AR, Cook DG, Bush T, Stedman JR, et al. Chronic exposure to outdoor air pollution and diagnosed cardiovascular disease: meta-analysis of three large cross-sectional surveys. Environ Health. 2009;8:30-8.

81. Lee D-H, Steffes MW, Sjödin A, Jones RS, Needham LL, Jacobs DRJ. Low dose organochlorine pesticides and polychlorinated biphenyls predict obesity, dyslipidemia, and insulin resistance among people free of diabetes. PlosOne. 2011;6(1):e15977.

82. Jones OAH, Maguire ML, Griffin JL. Environmental pollution and diabetes: a neglected association. Lancet. 2008;371:287-8.

83. Lee D-H, Lee I-K, Song K, Steffes M, Toscano W, Baker BA, et al. A strong dose-response relation between serum concentrations of persistent organic pollutants and diabtes. Diabetes Care. 2006;29(7):1638-44.

84. Ha M-H, Lee D-H, Jacobs DRJ. Association between serum concentrations or persistent organic pollutants and self-reported cardiovascular disease prevalence: results from National Health and Nutrition Examination Survey, 1999-2002. Environ Health Perspec. 2007;115:1204-9.

85. Esteban M, Castaño A. Non-invasive matrices in human biomonitoring: a review. Environ Int. 2009;35:438-49.

86. Aylward LL, Hays SM, Smolders R, Koch HM, Cocker J, Jones K, et al. Sources of variability in biomarker concentrations. J Toxicol Environ Health B. 2014;17:45-61.

87. Attflied KR, Hughes MD, Spengler JD, Lu C. Within- and between-child variation in repeated urinary pesticide metabolite measurements over a 1-year period. Environ Health Perspec. 2014;122(2):201-6.

88. Appenzeller BMR, Tsatsakis AM. Hair analysis for biomonitoring of environmental and occupational exposure to organic pollutants: State of the art, critical review and future needs. Toxicol Lett. 2012;210(2):119-40.

89. Salquebre G, Schummer C, Millet M, Briand O, Appenzeller BMR. Multi-class pesticide analysis in human hair by gas chromatography tandem (triple quadrupole) mass spectrometry with solid phase microextraction and liquid injection. Anal Chim Acta. 2012;710:65-74.
90. Schummer C, Appenzeller BMR, Millet M, Wennig R. Determination of hydroxylated metabolites of polycyclic aromatic hydrocarbons in human hair by gas chromatography-negative chemical ionization mass spectrometry. J Chromatogr A. 2009;1216(32):6012-9.
91. Appenzeller BMR, Mathon C, Schummer C, Alkerwi A, Lair M-L. Simultaneous determination of nicotine and PAH metabolites in human hair specimen: a potential methodology to assess tobacco smoke contribution in PAH exposure. Toxicol Lett. 2012;210(2):211-9.
92. Schummer C, Salquebre G, Briand O, Millet M, Appenzeller BMR. Determination of farm workers' exposure to pesticides by hair analysis. Toxicol Lett. 2012;210(2):203-10.
93. Raeppel C, Nief M, Fabritius M, Racault L, Appenzeller BMR, Millet M. Simultaneous analysis of pesticides from different chemical classes by using derivatisation step and gas chromatography-mass spectrometry. J Chromatogr A. 2011;1218:8123-9.
94. Kortenkamp A. Ten years of mixing cocktails: a review of combination effects of endocrine-disrupting chemicals. Environ Health Perspec. 2007;115(S1):98-105.
95. Reffstrup TK, Larsen JC, Meyer O. Risk assessment of mixtures of pesticides. Current approaches and future strategies. Regul Toxicol Pharm. 2010;56:174-92.
96. Fozard JL, Metter EJ, Brant LJ. Next steps in describing aging and disease in longitudinal studies. J Gerontol. 1990;45(4):P116-27.
97. Waldstein SR, Manuck SB, Ryan CM, Muldoon MF. Neuropsychological correlates of hypertension: review and methodologic considerations. Psychological bulletin. 1991;110(3):451-68.
98. Chamberlain SR, Odlaug BL, Schreiber LR, Grant JE. Association between tobacco smoking and cognitive functioning in young adults. Am J Addict. 2012;21 Suppl 1:S14-9.
99. Riggs KM, Spiro A, 3rd, Tucker K, Rush D. Relations of vitamin B-12, vitamin B-6, folate, and homocysteine to cognitive performance in the Normative Aging Study. The American journal of clinical nutrition. 1996;63(3):306-14.
100. Waldstein SR, Elias MF. Introduction to the special section on health and cognitive function. Health psychology : official journal of the Division of Health Psychology, American Psychological Association. 2003;22(6):555-8.

101. Watson NL, Sutton-Tyrrell K, Rosano C, Boudreau RM, Hardy SE, Simonsick EM, et al. Arterial stiffness and cognitive decline in well-functioning older adults. J Gerontol A Biol Sci Med Sci. 2011;66(12):1336-42.
102. Johnson TE. Recent results: biomarkers of aging. Exp Gerontol. 2006;41(12):1243-6.
103. Elias MF, Robbins MA, Budge MM, Abhayaratna WP, Dore GA, Elias PK. Arterial pulse wave velocity and cognition with advancing age. Hypertension. 2009;53(4):668-73.
104. Benetos A, Waeber B, Izzo J, Mitchell G, Resnick L, Asmar R, et al. Influence of age, risk factors, and cardiovascular and renal disease on arterial stiffness: clinical applications. Am J Hypertens. 2002;15(12):1101-8.
105. Schiffrin EL. Vascular stiffening and arterial compliance. Implications for systolic blood pressure. Am J Hypertens. 2004;17(12 Pt 2):39S-48S.
106. Waldstein SR, Rice SC, Thayer JF, Najjar SS, Scuteri A, Zonderman AB. Pulse pressure and pulse wave velocity are related to cognitive decline in the Baltimore Longitudinal Study of Aging. Hypertension. 2008;51(1):99-104.
107. Mitchell GF. Effects of central arterial aging on the structure and function of the peripheral vasculature: implications for end-organ damage. J Appl Physiol (1985). 2008;105(5):1652-60.
108. Henskens LH, Kroon AA, van Oostenbrugge RJ, Gronenschild EH, Fuss-Lejeune MM, Hofman PA, et al. Increased aortic pulse wave velocity is associated with silent cerebral small-vessel disease in hypertensive patients. Hypertension. 2008;52(6):1120-6.
109. Zeki Al Hazzouri A, Newman AB, Simonsick E, Sink KM, Sutton Tyrrell K, Watson N, et al. Pulse wave velocity and cognitive decline in elders: the Health, Aging, and Body Composition study. Stroke. 2013;44(2):388-93.
110. Arntzen KA, Schirmer H, Wilsgaard T, Mathiesen EB. Impact of cardiovascular risk factors on cognitive function: the Tromso study. Eur J Neurol. 2011;18(5):737-43.
111. Kivimaki M, Ferrie JE. Epidemiology of healthy ageing and the idea of more refined outcome measures. International journal of epidemiology. 2011;40(4):845-7.
112. Hogan DB, MacKnight C, Bergman H. Models, definitions, and criteria of frailty. Aging clinical and experimental research. 2003;15(3 Suppl):1-29.
113. Cawthon PM, Marshall LM, Michael Y, Dam TT, Ensrud KE, Barrett-Connor E, et al. Frailty in older men: prevalence, progression, and relationship with mortality. J Am Geriatr Soc. 2007;55(8):1216-23.
114. Fuchs J, Scheidt-Nave C, Hinrichs T, Mergenthaler A, Stein J, Riedel-Heller SG, et al. Indicators for Healthy Ageing -- A Debate. International journal of environmental research and public health. 2013;10(12):6630-44.

115. Elbaz A, Sabia S, Brunner E, Shipley M, Marmot M, Kivimaki M, et al. Association of walking speed in late midlife with mortality: results from the Whitehall II cohort study. Age (Dordrecht, Netherlands). 2013;35(3):943-52.
116. Batty GD, Shipley MJ, Kivimaki M, Marmot M, Davey Smith G. Walking pace, leisure time physical activity, and resting heart rate in relation to disease-specific mortality in London: 40 years follow-up of the original Whitehall study. An update of our work with professor Jerry N. Morris (1910-2009). Annals of epidemiology. 2010;20(9):661-9.
117. Koopman JJ, van Bodegom D, van Heemst D, Westendorp RG. Handgrip strength, ageing and mortality in rural Africa. Age and ageing. 2014.
118. Riordan JR. Cystic fibrosis as a disease of misprocessing of the cystic fibrosis transmembrane conductance regulator glycoprotein. American journal of human genetics. 1999;64(6):1499-504.
119. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. Cell. 1993;72(6):971-83.
120. International HapMap C. A haplotype map of the human genome. Nature. 2005;437(7063):1299-320.
121. International HapMap C, Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, et al. A second generation human haplotype map of over 3.1 million SNPs. Nature. 2007;449(7164):851-61.
122. Wellcome Trust Case Control C. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007;447(7145):661-78.
123. Hindorff LA, MacArthur J, Morales J, Junkins HA, Hall PN, Klemm AK, et al. A Catalog of Published Genome-Wide Association Studies. Available at: wwwgenomegov/gwastudies Accessed [03/02/2014].
124. Direction de la Santé DoH, Ministry of Health, Luxembourg. National Statistics of all causes of death, Statistiques des causes de décès, . 2010.
125. Alkerwi A, Sauvageot N, Donneau AF, Lair ML, Couffignal S, Beissel J, et al. First nationwide survey on cardiovascular risk factors in Grand-Duchy of Luxembourg (ORISCAV-LUX). BMC public health. 2010;10:468.
126. Alkerwi A, Sauvageot N, Couffignal S, Albert A, Lair ML, Guillaume M. Comparison of participants and non-participants to the ORISCAV-LUX population-based study on cardiovascular risk factors in Luxembourg. BMC medical research methodology. 2010;10:80.

127. Alkerwi A, Donneau AF, Sauvageot N, Lair ML, Scheen A, Albert A, et al. Prevalence of the metabolic syndrome in Luxembourg according to the Joint Interim Statement definition estimated from the ORISCAV-LUX study. BMC public health. 2011;11(1):4.
128. Alkerwi A, Pagny S, Lair ML, Delagardelle C, Beissel J. Level of unawareness and management of diabetes, hypertension, and dyslipidemia among adults in Luxembourg: findings from ORISCAV-LUX study. PLoS One. 2013;8(3):e57920.
129. Alkerwi A, Sauvageot N, Pagny S, Beissel J, Delagardelle C, Lair ML. Acculturation, immigration status and cardiovascular risk factors among Portuguese immigrants to Luxembourg: findings from ORISCAV-LUX study. BMC public health. 2012;12:864.
130. Ford ES, Kohl HW, 3rd, Mokdad AH, Ajani UA. Sedentary behavior, physical activity, and the metabolic syndrome among U.S. adults. Obes Res. 2005;13(3):608-14.
131. Liese AD, Mayer-Davis EJ, Haffner SM. Development of the multiple metabolic syndrome: an epidemiologic perspective. Epidemiol Rev. 1998;20(2):157-72.
132. Alkerwi A, Donneau AF, Sauvageot N, Lair ML, Albert A, Guillaume M. Dietary, behavioural and socio-economic determinants of the metabolic syndrome among adults in Luxembourg: findings from the ORISCAV-LUX study. Public Health Nutr. 2011:1-11.
133. Diet, nutrition and the prevention of chronic diseases. World Health Organ Tech Rep Ser. 2003;916:i-viii, 1-149, backcover.
134. Alkerwi A, Sauvageot N, Nau A, Lair ML, Donneau AF, Albert A, et al. Population compliance with national dietary recommendations and its determinants: findings from the ORISCAV-LUX study. Br J Nutr. 2012:1-10.
135. Alkerwi A, Crichton GE, Hebert JR. Consumption of ready-made meals and increased risk of obesity: findings from the Observation of Cardiovascular Risk Factors in Luxembourg (ORISCAV-LUX) study. Br J Nutr. 2014:1-8.
136. Crichton GE, Alkerwi A. Whole-fat dairy food intake is inversely associated with obesity prevalence: findings from the Observation of Cardiovascular Risk Factors in Luxembourg study. Nutrition research. 2014;34(11):936-43.
137. Holmberg S, Thelin A. High dairy fat intake related to less central obesity: a male cohort study with 12 years' follow-up. Scand J Prim Health Care. 2013;31(2):89-94.
138. Crichton GE, Alkerwi A. Dairy food intake is positively associated with cardiovascular health: findings from Observation of Cardiovascular Risk Factors in Luxembourg study. Nutrition research. 2014;34(12):1036-44.
139. Alkerwi A, Sauvageot N, Buckley JD, Donneau AF, Albert A, Guillaume M, et al. The potential impact of animal protein intake on global and abdominal obesity: evidence from the

Observation of Cardiovascular Risk Factors in Luxembourg (ORISCAV-LUX) study. Public health nutrition. 2015:1-8.

140. Alkerwi A, Vernier C, Crichton GE, Sauvageot N, Shivappa N, Hebert JR. Cross-comparison of diet quality indices for predicting chronic disease risk: findings from the Observation of Cardiovascular Risk Factors in Luxembourg (ORISCAV-LUX) study. The British journal of nutrition. 2014:1-11.

141. Crichton GE, Alkerwi A. Association of sedentary behavior time with ideal cardiovascular health: the ORISCAV-LUX study. PLoS One. 2014;9(6):e99829.

142. Healy GN, Clark BK, Winkler EA, Gardiner PA, Brown WJ, Matthews CE. Measurement of adults' sedentary time in population-based studies. Am J Prev Med. 2011;41(2):216-27.

143. Crichton GE, Elias MF, Davey A, Alkerwi A. Cardiovascular health and cognitive function: the Maine-Syracuse Longitudinal Study. PLoS One. 2014;9(3):e89317.

144. Goretti E, Vausort M, Wagner DR, Devaux Y. Association between circulating microRNAs, cardiovascular risk factors and outcome in patients with acute myocardial infarction. International journal of cardiology. 2013;168(4):4548-50.

145. Vausort M, Wagner DR, Devaux Y. Long noncoding RNAs in patients with acute myocardial infarction. Circulation research. 2014;115(7):668-77.

146. Goretti E, Wagner DR, Devaux Y. miRNAs as biomarkers of myocardial infarction: a step forward towards personalized medicine? Trends in molecular medicine. 2014;20(12):716-25.

147. Wagner DR, Devaux Y, Collignon O. Door-to-balloon time and mortality. N Engl J Med. 2014;370(2):180-1.

148. Appenzeller BM, Mathon C, Schummer C, Alkerwi A, Lair ML. Simultaneous determination of nicotine and PAH metabolites in human hair specimen: a potential methodology to assess tobacco smoke contribution in PAH exposure. Toxicology letters. 2012;210(2):211-9.

149. Vartiainen E, Sarti C, Tuomilehto J, Kuulasmaa K. Do changes in cardiovascular risk factors explain changes in mortality from stroke in Finland? Bmj. 1995;310(6984):901-4.

150. Report WHO. Action plan for the global strategy for the prevention and control of non communicable diseases 2008-2013. Report by the director General. 2008 2008. Report No.

151. Ford ES, Li C, Zhao G, Pearson WS, Capewell S. Trends in the prevalence of low risk factor burden for cardiovascular disease among United States adults. Circulation. 2009;120(13):1181-8.

152. Butler RN, Miller RA, Perry D, Carnes BA, Williams TF, Cassel C, et al. New model of health promotion and disease prevention for the 21st century. Bmj. 2008;337:a399.
153. Devaux Y, Vausort M, Goretti E, Nazarov PV, Azuaje F, Gilson G, et al. Use of circulating microRNAs to diagnose acute myocardial infarction. Clinical chemistry. 2012;58(3):559-67.
154. Samouda H, Dutour A, Chaumoitre K, Panuel M, Dutour O, Dadoun F. VAT=TAAT-SAAT: innovative anthropometric model to predict visceral adipose tissue without resort to CT-Scan or DXA. Obesity. 2013;21(1):E41-50.
155. BODY COMPOSITION ANALYZER BC-418 INSTRUCTION MANUAL. http://www.tanita.com/en/bc-418/.
156. Volgyi E, Tylavsky FA, Lyytikainen A, Suominen H, Alen M, Cheng S. Assessing body composition with DXA and bioimpedance: effects of obesity, physical activity, and age. Obesity. 2008;16(3):700-5.
157. Knight E, Stuckey MI, Petrella RJ. Validation of the step test and exercise prescription tool for adults. Canadian journal of diabetes. 2014;38(3):164-71.
158. Villars C, Bergouignan A, Dugas J, Antoun E, Schoeller DA, Roth H, et al. Validity of combining heart rate and uniaxial acceleration to measure free-living physical activity energy expenditure in young men. Journal of applied physiology (Bethesda, Md : 1985). 2012;113(11):1763-71.
159. van Hees VT, Fang Z, Langford J, Assah F, Mohammad A, da Silva IC, et al. Autocalibration of accelerometer data for free-living physical activity assessment using local gravity and temperature: an evaluation on four continents. Journal of applied physiology. 2014;117(7):738-44.
160. Sabia S, van Hees VT, Shipley MJ, Trenell MI, Hagger-Johnson G, Elbaz A, et al. Association between questionnaire- and accelerometer-assessed physical activity: the role of sociodemographic factors. American journal of epidemiology. 2014;179(6):781-90.
161. da Silva IC, van Hees VT, Ramires VV, Knuth AG, Bielemann RM, Ekelund U, et al. Physical activity levels in three Brazilian birth cohorts as assessed with raw triaxial wrist accelerometry. International journal of epidemiology. 2014;43(6):1959-68.
162. National HES manual, EHES study, country Luxembourg. 2011.
163. Clark V. Sample size determination. Plastic and reconstructive surgery. 1991;87(3):569-73.
164. Samaropoulos XF, Hairston KG, Anderson A, Haffner SM, Lorenzo C, Montez M, et al. A metabolically healthy obese phenotype in hispanic participants in the IRAS family study. Obesity. 2013;21(11):2303-9.

165. Williams JR. The Declaration of Helsinki and public health. Bulletin of the World Health Organization. 2008;86(8):650-2.
166. ADELF. Déontologie et bonnes pratiques en épidémiologie. 2003.